Reviewer #1 (Remarks to the Author):

In this study, Homeira Moradi Chameh and colleagues characterize the electrophysiological properties of single neurons primarily located in layers 2/3 and 5 of the human anterior temporal lobe. The authors show that sag currents, a functional measure of h channel protein expression, contribute to inter-laminar differences that might have implications for network function and emergent properties and oscillations tied to fundamental aspects of brain function. It is also of interest that the sag currents correlated with patient age, and that these important findings could be replicated in a separate large dataset of human neuron recordings from the Allen Institute. While the study adds to our growing knowledge of human neuron functional properties and is indeed a sizable dataset, it is conspicuously descriptive in nature. That said, such studies are an important contribution to the field, given our limited knowledge of human brain cell type diversity and functional properties, and the rare opportunity to collect this type of data from surgery-derived specimens. Nonetheless, it stands that some methodological considerations (as described in detail below) limit the conclusions and new insights that can be drawn from the present work, and thus, dampen enthusiasm to some extent. In the present form, it is not clear that this work will be of high enough interest to the broad readership of Nature Communications, and it seems a better fit for a specialty journal in neurophysiology or cellular neuroscience. That said, it is possible that additional targeted experiments could be performed to address the concerns and strengthen the findings. It is important to publish rigorous results about human neuron properties if we are to uncover human-specific features of brain cell types and their contributions to health and disease.

Detailed comments:

1) The electrophysiology experiments are well-executed and the analysis of cellular features is sound. However, it seems potentially ill-conceived to treat layers in human neocortex as discrete groupings for aggregating data and comparison of features across layers. In particular, the human L2/3 is greatly expanded in size compared to mammals such as widely studied rodent L2/3, which is of high interest in evolutionary neuroscience and for understanding unique cognitive abilities of humans. A coarse inspection of histological stains or Golgi fills reveals a substantial heterogeneity and clear gradients of cell density, size, and morphologies throughout this region (see also Mohan et al, 2015 Cerebral Cortex; Deitcher et al, 2017 Cerebral Cortex; Kalmbach et al, 2018 Neuron). It is expected that such diversity relates to functional differences, and perhaps to discrete types (as recently described by Hodge et al, 2019 Nature based on gene expression and clustering methods). The worry is that aggregation of data from L2/3 (or 5) masks deeper diversity, and possibly functionally important distinctions that reside within. For example, significant differences in the F/I curves of two types of L2/3 human pyramidal neurons have been described in Deitcher et al, 2017 (not cited in this study), similar to the differences found between L2/3 vs L5 populations in Fig 3c here. Similarly, the differences found for sag and sag ratio for L2/3 vs L5 neurons in this study is of similar magnitude to differences reported for superficial L2 vs deeper L3 human pyramidal neurons in a previous study (Kalmbach et al, 2018). Although the author's note that the detailed position of recorded neurons within L2/3 depth was not recorded or appreciated (and partially addressed with minimal additional data in Fig S1), this stands as a significant down-side limiting the interpretation of the data.

2) Notably, the Beaulieu-Laroche et al 2018 Cell study also described similar values and differences in human L2/3 vs L5 pyramidal neurons, although not presented explicitly as comparisons across lamina (as the focus was on rat vs human comparisons in L5 and L2/3). The relevant data on L2/3 is

easily missed in the supplemental data. Regardless, these findings should be discussed and cited, and perhaps can be used to bolster the findings of the current study. Although the data itself may not be completely novel on human L2/3 and L5 pyramidal neuron electrophysiological properties, the direct comparisons across layers, loose connection to circuit properties and oscillations, and data interpretation in the present study is still novel.

3) The absence of strong evidence for (theta) resonance in either L5 or L2/3 pyramidal neurons from the chirp analysis is further suggestive of potential sampling bias in the dataset that could skew the interpretation of the data. The neuron types most likely to exhibit this cellular property (related to sag amplitude) are either found in the deepest part of L3c of human temporal cortex (discussed in Deitcher et al 2017), or are among the rarest L5 thick-tufted (presumed sub-cortically projecting) type (Beaulieu-Laroche et al, 2019; Hodge et al, 2019). Although admittedly this is not common knowledge from published studies, I mention here with the intent of stimulating further thought on this point, and whether additional targeted sampling (especially the magnopyramidal neurons in deep L3c) might help to clarify on this evidence and whether or not subpopulations in both layers do in fact exhibit strong subthreshold resonance. Targeted experiments are indeed helpful, as demonstrated by Beaulieu-Laroche et al, 2019, where rare thick-tufted L5 pyramidal neurons were recorded by targeting of their prominent apical dendrites, which can be readily distinguished by expert dendritic patchers. Clarification on potential sampling bias and due diligence on searching for these noted types would strengthen the interesting findings in Figs 4 and 5 of the present study.

4) Related to potential sampling bias and position of neurons in the cortical depth, in Fig 6, the correlations and regression lines will be heavily influenced by the exact distribution of depth from pia of the collective cells sampled for each donor. Although this can't be deconvolved at this stage, it should be mentioned as a caveat of the data interpretation.

5) There is not sufficient evidence presented to determine if the lack of subthreshold resonance in L2/3 (especially deep L3c) and L5 neurons might be the consequence of partial truncation of the apical dendrites. This is of interest to address in more detail because it is known that h channels that impart resonance are highly concentrated in the distal dendrites of L5 pyramidal neurons (see Beaulieu-Laroche et al, 2019). Certainly, the high range in the distribution of Rn values may suggest a proportion of cells with truncations, and this is a common and perhaps unavoidable issue in dealing with human pyramidal neurons that have apical dendrites up to 2 mm in length (see also Mohan et al, 2015 Cerebral Cortex). Given that dendritic truncation can also greatly impact bursting behavior of pyramidal neurons, it is probably important to at least mention this point, although clearly this did not impede detection of higher proportion of bursting neurons in human L5 vs L2/3 (but also previously described in Beaulieu-Laroche et al, 2019, which should be noted).

Reviewer #2 (Remarks to the Author):

The authors describe the differences in passive and active membrane properties of L2/3 and L5 pyramidal cells in human brain slices obtained mainly from the anterior temporal lobe of epilepsy patients. They found L5 PCs to have higher input resistance, more h-current, and higher gain in response to noisy inputs at delta to theta frequency range compared to L2/3 PCs. The authors propose that these differences in intrinsic properties may serve as the cellular underpinnings for the generation of theta oscillation at deeper layers and its subsequent propagation to more superficial layers, which they (and others) have reported previously.

The difference in h-current reported here is not surprising given that it is well established from rodent studies that neocortical L5 PCs, particularly the subcortically-projecting subpopulation, have large h-currents, while L2/3 PCs have little to none (van Aerde & Feldmeyer, 2015). This was also confirmed in human studes conducted from samples similar to what was used in the current manuscript (Kalmbach et al., 2018, Beaulieu-Laroche et al., 2018). Contribution of h-current to increased input impedance at the delta-theta frequency range and intrinsic burst firing has likewise been explored previously in both rodent and human studies (Hu et al., 2002; Kalmbach et al., 2018).

A strength of the study lies in its attempt to provide a mechanistic explanation for phenomena that have been observed at a more mesoscopic level in vivo in humans. However, the connection is weak, and most of the findings at the cellular level have already been reported previously from both rodent and human samples. The implied causal relationship between the stronger h-current in human L5 PCs and their increased responsiveness to theta input, despite some existing literature making this argument, is not immediately evident in the current manuscript to be conclusive. In addition, there are serious concerns regarding the electrophysiological data, methodology, and analysis.

Major points:

1. The overarching theme throughout the manuscript is that the prominence of h-current in human L5 PCs endows them with increased responsiveness to delta to theta frequency inputs as well as intrinsic bursting properties. However, it is unclear that the h-current is responsible for the generation of theta oscillation by influencing the frequency-dependent gain of each cell type, especially considering that the authors report L5 PCs to have higher input resistance and slower membrane time constant compared with L2/3 PCs. It appears that, as a minimum, repeating the frequency-dependent gain analysis in the presence of ZD7288 would be necessary to support the authors' claim in this context.

2. While the criteria are not agreed upon and the terminology is diverse, it is widely accepted that neocortical L5 pyramidal neurons fall largely into at least two major cell types, with distinguishable long-range projection targets, morphology, and membrane properties, including the size of the h-current (Dembrow et al., 2010). The two different subpopulations also exhibit contrasting local and long-range connectivity, at least in different neocortical regions of rodents (Morishima & Kawaguchi, 2006; Morishima et al., 2011; Morishima et al., 2017; Collins et al., 2018; Anastasiades et al., 2018). Additionally, emerging evidence suggests the presence of cell-type specific properties amongst L2/3 neurons specifically in human slices (Kalmbach et al., 2018), specifically that superficial neurons have little to no h-current and deeper neurons have significant amounts. In light of these points, it is unclear why the authors have decided to group all their recordings into L5 vs L2/3. This is a major potential confound for the authors' interpretation that could be addressed with some straightforward classification and/or clustering.

3. Much of the electrophysiological data presented in the current manuscript are hard to reconcile with previous studies. First, L2/3 PCs were reported to have higher input resistance compared with L5 PCs, in both rodents and in humans (Bitzenhofer et al., 2017; Beaulieu-Laroche et al., 2018; Larkum & Zhu, 2002; Larkum et al., 2007), even though reports in the opposite direction are also present from different authors, albeit not within a single paper (Chang et al., 2005; Luebke et al.,

2007). The discrepancy in terms of input resistance may be, however, due simply to technical differences as different groups tend to have different definitions of input resistance and voltage sag in the presence of h-current. It would therefore be helpful if the authors stated this clearly in their methods and discussion the many substantial disagreements with the published literature. Second, the gain of the current-frequency relationships in Fig. 3C also appear to be higher compared to previous observations, with L2/3 cells already saturating at +350 pA injection. Third, a previous study by Beaulieu-Laroche et al. reported an absence in correlation between patient age and dendritic h-current (albeit with a smaller sample number), which disagrees with one of the major points of the current manuscript. In addition, the correlations the authors report may be subject to methodological and/or statistical problems: despite the massive number of neurons in the Allen Institute dataset for L2/3 neurons, there is essentially no trend, in contrast to the authors' L2/3 data. The authors' L2/3 correlation (Fig. 6C) appears to be strongly driven by a surprising disconnect between the second-oldest patient group in the mid-40s and the oldest patient group of what appears to be early-50s. How is this kind of sampling possible? If the authors left this conspicuous older group out, there would be no correlation across 20 years of patient data. Similarly, the strong correlation in Fig. 6A appears driven by a relatively few neurons from 20-25 year olds with low (even negative!) sag ratios, which seems highly implausible. As does the three 80+ year old patients with extremely high sag ratios in the Allen Institute database. These kinds of correlational analyses are fraught with potential confounds, one of which is likely the sampling of different cell types in the different patients. The authors need to do a much more rigorous and principled job of dealing with these data than just blindly fitting scatterplots.

4. The data in the manuscript exhibits a surprising degree of variance, potentially coming from poor experimental quality control and/or inappropriate analyses. For example, Fig. 1 B-D shows enormous ranges for values that are generally agreed upon to be very tightly controlled in a given cell type. A 20 to 30 mV range in resting potential for pyramidal neurons is completely unprecedented and does correspond to the vast majority of previous studies. The same goes for input resistance and membrane time constant. The authors must either explain this remarkable variance or cluster/categorize/curate their data according to previously established criteria.

5. References are placed in inappropriate contexts. As an example, the authors reference four papers by Sakmann and Petersen groups in support of their statement that L5 PCs are more excitable than L2/3 cells (page 20). However, all of these papers are exclusively about L2/3 PCs, and none of them mention L5 PCs (except a couple of deeper-positioned cells in the supplement for one of the papers), let alone their input resistances. Furthermore, all of these experiments were conducted in vivo, wherein synaptic conductances will reduce the apparent input resistance of the cell (Destexhe et al., 2001), making direct comparisons with in vitro studies from slices inappropriate. As another example, the authors point to Larsen et al. (2008) to describe the connection properties of intrinsically bursting cells, but said paper is strictly structural and does not discuss electrophysiology. This issue needs to be addressed.

6. The authors state that they used paired or unpaired t-tests (page 7), except for frequency-dependent gain analysis. The majority of the data, however, are obviously not in normal distribution. Nonparametric tests would therefore be more appropriate.

7. Whole-cell voltage-clamp is not quantitative in neurons with dendrites and voltage-dependent ion channels. It is not clear what the data in G-I add to the manuscript. It should be removed.

Minor points:

1. The depths of the cell bodies for the reconstructed cells in Fig. 1A don't correspond to the anatomical images: are L2/3 and L5 PCs offset from one another, or on different scales? The authors should show laminar borders for these reconstructions if that is how they define L2/3 vs L5. A further anatomical point: are the authors concerned that what they are calling L5 PCs appear to have no apical tuft dendrites?

2. The authors describe that "the half-width of the action potential was shorter in L5 pyramidal cells compared to L2/3 pyramidal cells" (page 13). The associated p-value, however, is 0.07 and should be considered nonsignificant. Either correct the p-value or rephrase the statement.

3. From the solution compositions in methods, it would be more likely for the liquid junction potential to be - 10.8 mV, instead of the + 10.8 mV as appears on the manuscript (page 4).

4. "The membrane time constant (tau_m) was calculated using a single-exponential fit of the membrane potential response to a small hyperpolarizing pulse." (page 4): what was the amplitude of the hyperpolarizing current injection (instead of "small")?

5. "I_h appeared to be one of the major contributors to the prominent post-inhibitory depolarization" (page 20): Hyperpolarizing current injection is not "inhibitory" per se.

6. The definitions of stimulus-response correlation (Csr) and stimulus-stimulus autocorrelation (Css) are not given in the methods (page 6). They are given in the referenced Higgs & Spain paper.

7. Referring to the resonance frequency in the impedance amplitude profile formed by h-current as "low frequency membrane resonance" (page 15) sounds misleading because the effects of the h-current is to impose a high-pass filter. Perhaps rephrasing this could be better.

8. There are numerous typographical errors throughout the manuscript that pose as distractions for the reader. Some, but not all, examples include: Repeated occurrences of "*, **,***, indicate significantly different at P < 0.001 , P < 0.05, P < 0.001" (in legends), "L5: 68 ± 8mV" (page 9, describing RMP), "lager sag" (page 10), "we also we performed" (page 11), "we surprisingly we found" (page 15), "Wilcox test" (page 18), "I_Nap" (page 20, with undercase p), "Hu et. al" (page 21), and initials of the first author (page 22).

Reviewer #3 (Remarks to the Author):

This is a thorough, careful and thoughtful MS quantifying the distribution of Ih in human – mainly anterior temporal – neocortex by layer. I completely concur with the ethos behind the study – that modelling the human brain demands constraint by human data and, for this reason alone, the paper constitutes a valuable reference work and so to me, deserves publication.
I do have a couple of issues with the MS as it stands though. The comments below are, hopefully, easily dealt with and are intended merely as suggestions to improve the MS.
1) I see why the authors 'hang' their findings on the human theta rhythm. But I don't think its valid

given precedents in rodent literature. As they argue in the discussion, there are multiple mechanisms that can underlie neuronal rhythmicity at theta frequency. Ih is almost certainly not one of them in neocortex. Hippocampal theta cannot reliably be considered the same phenomenon, the frequencies are different for a start. Here there is a dependence on Ih but it lies principally in the behaviour of a subset of interneurons and the neocortical equivalents were not examined in the present MS. In addition, Ih is exquisitely dependent on neuromodulatory state: Theta is mainly seen in non-invasive human recordings in the wake state but, in this condition, multiple wake-associated neuromodulators all act to reduce Ih considerably (e.g. Ach, Orexin/hypocretin etc.). What Ih IS critically involved in is rebound following synaptic inhibition. Thus it plays a crucial role in delayed responses to sensory input (the 'off' response) and 'anodal break' spiking seen in mismatch responses.

2) The data is presented with commendable clarity, but consequently suggests multimodal distributions in a number of the intrinsic cell properties measured. This is discussed briefly (L2 vs L3 for example) but the main subdivision of cell type I know of that manifests in part as sag amplitude differences is between L5a and L5b. The authors have gathered an impressive set of data so I wonder if it is possible to stratify L2/3 and L5 cell types further on the basis of other intrinsic properties (slow AHP, burst generation, afterdepolarisation strength etc.) This may help to 'clean up' the often very broad distributions in some of the metrics.

2 minor points:

3) Lack of observed resonance on somatic recordings with patch electrodes is not surprising. Patch solutions dialyse cytosol hugely and thus interfere with many intrinsic conductances. In addition, the distribution of Ih in neurons is not uniform and resonance can be seen in dendritic recordings in a given cell type when it appears completely absent in somatic recording.

4) The MS data does agree with the Carracedo data in terms of layers showing most theta (discussion). In that paper the field theta was mainly manifest in superficial layers but the origin of the synaptic activity underlying this was exclusively intrinsic theta activity in a subpopulation of L5 cells. See point 1 above though, this theta was not Ih-dependent.

1

2 Reviewers' comments:

3 Reviewer#1 (Remarks to the Author):

4 In this study, Homeira Moradi Chameh and colleagues characterize the electrophysiological
5 properties of single neurons primarily located in layers 2/3 and 5 of the human anterior temporal
6 lobe. The authors show that sag currents, a functional measure of h channel protein expression,
7 contribute to inter-laminar differences that might have implications for network function and
8 emergent properties and oscillations tied to fundamental aspects of brain function. It is also of
9 interest that the sag currents correlated with patient age, and that these important findings
10 could be replicated in a separate large dataset of human neuron recordings from the Allen
11 Institute. While the study adds to our growing knowledge of human neuron functional properties
12 and is indeed a sizable dataset, it is conspicuously descriptive in nature. That said, such studies
13 are an important contribution to the field, given our limited knowledge of human brain cell type.

14 Diversity and functional properties; and the rare opportunity to collect this type of data from
15 surgery-derived specimens. Nonetheless, it stands that some methodological considerations (as
16 described in detail below) limit the conclusions and new insights that can be drawn from the
17 present work, and thus, dampen enthusiasm to some extent. In the present form, it is not clear
18 that this work will be of high enough interest to the broad readership of Nature Communications,
19 and it seems a better fit for a specialty journal in neurophysiology or cellular neuroscience. That
20 said, it is possible that additional targeted experiments could be performed to address the
21 concerns and strengthen the findings. It is important to publish rigorous results about human
22 neuron properties if we are to uncover human-specific features of brain cell types and their
23 contributions to health and disease.

24

25 Detailed comments:

26

27 1) The electrophysiology experiments are well-executed, and the analysis of cellular features is
28 sound. However, it seems potentially ill-conceived to treat layers in human neocortex as discrete
29 groupings for aggregating data and comparison of features across layers. In particular, the
30 human L2/3 is greatly expanded in size compared to mammals such as widely studied rodent
31 L2/3, which is of high interest in evolutionary neuroscience and for understanding unique
32 cognitive abilities of humans. A coarse inspection of histological stains or Golgi fills reveals a
33 substantial heterogeneity and clear gradients of cell density, size, and morphologies throughout
34 this region (see also Mohan et al, 2015 Cerebral Cortex; Deitcher et al, 2017 Cerebral Cortex;
35 Kalmbach et al, 2018 Neuron). It is expected that such diversity relates to functional differences,
36 and perhaps to discrete types (as recently described by Hodge et al, 2019 Nature based on gene
37 expression and clustering methods). The worry is that aggregation of data from L2/3 (or 5) masks
38 deeper diversity, and possibly functionally important distinctions that reside within. For
39 example, significant differences in the F/I curves of two types of L2/3 human pyramidal neurons

40 have been described in Deitcher et al, 2017 (not cited in this study), similar to the differences
41 found between L2/3 vs L5 populations in Fig 3c here. Similarly, the differences found for sag and
42 sag ratio for L2/3 vs L5 neurons in this study is of similar magnitude to differences reported for
43 superficial L2 vs deeper L3 human pyramidal neurons in a previous study (Kalmbach et al, 2018).
44 Although the author's note that the detailed position of recorded neurons within L2/3 depth was
45 not recorded or appreciated (and partially addressed with minimal additional data in Fig S1), this
46 stands as a significant downside limiting the interpretation of the data.

47 We thank the reviewer for articulating this concern and note that each of the reviewers raised a
48 similar issue with our prior submission.

49 We have addressed this concern in our re-submission:

50 First, as suggested by this reviewer, we have now conducted a targeted set of recordings of deep
51 layer 3c pyramidal cells (at 1100-1400 μm distance from pia), allowing us to better parse the
52 heterogeneity inherent in our prior layer 2/3 pyramidal cell recordings. These recordings allowed
53 us to replicate prior findings from Kalmbach, B et al (2018) showing that sag amplitudes increase
54 as a function of depth within superficial cortical layers in human cortex (Fig 2B) and that L3c
55 pyramidal cells are more likely to be resonant (Fig 4).

56 Second, we now present in Fig 6 an analysis of cell type sub-clusters and gradients based on
57 multi-variate correlations in pyramidal cells' basic electrophysiology features, such as input
58 resistance, resting potential, action potential width at rheobase, etc., with an attempt to connect
59 these gradients to cell morphologies (where available). This analysis suggested that, as described
60 previously, there exists a great degree of heterogeneity within each of the cellular layers
61 sampled here, with cells sampled from L2/3 and Layer 3c often displaying similar
62 electrophysiological features to those from Layer 5 pyramidal cells. This analysis corroborates
63 the reviewer's comment and considerable previous work suggesting that there is considerable
64 heterogeneity within cells sampled from each layer (Berg, Sorensen et al. 2020), with much of
65 this variability being correlated with morphological differences (such as those shown in
66 (Deitcher, Eyal et al. 2017), and that this is comparable to (or even greater than)
67 electrophysiological differences across layers.

68

69 2) Notably, the Beaulieu-Laroche et al 2018 Cell study also described similar values and
70 differences in human L2/3 vs L5 pyramidal neurons, although not presented explicitly as
71 comparisons across lamina (as the focus was on rat vs human comparisons in L5 and L2/3). The
72 relevant data on L2/3 is easily missed in the supplemental data. Regardless, these findings should
73 be discussed and cited, and perhaps can be used to bolster the findings of the current study.
74 Although the data itself may not be completely novel on human L2/3 and L5 pyramidal neuron
75 electrophysiological properties, the direct comparisons across layers, loose connection to circuit
76 properties and oscillations, and data interpretation in the present study is still novel.

77 We sincerely thank the reviewer for highlighting this recent paper that is highly relevant to our
78 study. While we had cited this paper previously, not including a more thorough comparison of
79 our data to that from this work was a major oversight on our part in our prior submission. In the
80 revised manuscript, we have attempted to compare and contrast our findings with those from
81 (Beaulieu-Laroche, Toloza et al. 2018).

82 In brief, we feel that our findings are mostly consistent with those reported in Beaulieu-Laroche,
83 L et al (2018), with the caveat that the data in Beaulieu-Laroche, L et al (2018) were targeted
84 towards larger, thick-tufted cells in L5, whereas our data from L5 are likely a mix of thin- and
85 thick-tufted cells (with a suspected bias towards thin-tufted cells). To the extent that there are
86 differences between our studies, for example, that Beaulieu-Laroche, L et al (2018) report lower
87 cellular input resistances in L5 relative to L2/3 whereas we do not, our hypothesis is that this
88 difference in sampling strategies likely underlie such differences.

89 3) The absence of strong evidence for (theta) resonance in either L5 or L2/3 pyramidal neurons
90 from the chirp analysis is further suggestive of potential sampling bias in the dataset that could
91 skew the interpretation of the data. The neuron types most likely to exhibit this cellular property
92 (related to sag amplitude) are either found in the deepest part of L3c of human temporal cortex
93 (discussed in Deitcher et al 2017), or are among the rarest L5 thick-tufted (presumed sub-
94 cortically projecting) type (Beaulieu-Laroche et al, 2019; Hodge et al, 2019). Although admittedly
95 this is not common knowledge from published studies, I mention here with the intent of
96 stimulating further thought on this point, and whether additional targeted sampling (especially
97 the magnopyramidal neurons in deep L3c) might help to clarify on this evidence and whether or
98 not subpopulations in both layers do in fact exhibit strong subthreshold resonance. Targeted
99 experiments are indeed helpful, as
100 demonstrated by Beaulieu-Laroche et al, 2019, where rare thick-tufted L5 pyramidal neurons
101 were recorded by targeting of their prominent apical dendrites, which can be readily
102 distinguished by expert dendritic patchers. Clarification on potential sampling bias and due
103 diligence on searching for these noted types would strengthen the interesting findings in Figs 4
104 and 5 of the present study.

105 We thank this reviewer (and a helpful Skype call with Brian Kalmbach) for guidance on how to
106 better target pyramidal cells that are likely to display subthreshold resonance. In new data
107 presented within our resubmission, we have now targeted recordings to a subset of pyramidal
108 cells in human layer 3c. In addition, we have re-analyzed our prior ZAP/chirp data, taking care to
109 more carefully quantify subthreshold resonance at the "cell-level", as opposed to the layer level
110 as in our prior submission.
111
112 In our new analysis (Fig 4), we have now found a modest number of cells in each cortical layer
113 that display subthreshold resonance. We note, however, that the incidence of subthreshold
114 resonance at "theta" frequencies (> 2Hz) was still quite rare in our dataset, especially compared
115 to recent reports from Beaulieu-Laroche, L et al (2018) and Kalmbach, B et al (2018). It is certainly
116 possible that inadvertent confounds, such as dendrite truncation or cytosol dialysis (suggested

by reviewer #3), might obscure the greater expected prevalence of subthreshold resonance in these data. In the revised manuscript, we have now added text to our discussion (in the "limitations" subsection) contextualizing our findings and stating these possible sampling and truncation issues more clearly.

4) Related to potential sampling bias and position of neurons in the cortical depth, in Fig 6, the correlations and regression lines will be heavily influenced by the exact distribution of depth from pia of the collective cells sampled for each donor. Although this can't be deconvolved at this stage, it should be mentioned as a caveat of the data interpretation.

We thank the reviewer for pointing out this important issue of sampling bias and cortical depth as a potential confound in our analysis of Fig 6 (now Fig 8). We have now included further text in this section pointing out this confound.

5) There is not sufficient evidence presented to determine if the lack of subthreshold resonance in L2/3 (especially deep L3c) and L5 neurons might be the consequence of partial truncation of the apical dendrites. This is of interest to address in more detail because it is known that h channels that impart resonance are highly concentrated in the distal dendrites of L5 pyramidal neurons (see Beaulieu-Laroche et al, 2019). Certainly, the high range in the distribution of Rn values may suggest a proportion of cells with truncations, and this is a common and perhaps unavoidable issue in dealing with human pyramidal neurons that have apical dendrites up to 2 mm in length (see also Mohan et al, 2015 Cerebral Cortex). Given that dendritic truncation can also greatly impact bursting behavior of pyramidal neurons, it is probably important to at least mention this point, although clearly this did not impede detection of higher proportion of bursting neurons in human L5 vs L2/3 (but also previously described in Beaulieu-Laroche et al, 2019, which should be noted).

This is a fair point. It's certainly possible that the lack of observation of more subthreshold resonance is impacted by inadvertent truncation of dendrites. The high range in cell input resistances reported here would also support the observation that some of this range is likely due to truncation (see new supplemental figure S7 for further corroboration on this point using data from the Allen Institute where apical dendrite truncation was more systematically annotated). We have now taken care to mention the confound of dendrite truncation when reporting our results regarding input resistances and resonance.

Regarding the point about bursting, we have re-analyzed our data on bursting to use a definition of bursting that is inspired by that used in Beaulieu-Laroche, L et al (2018) (i.e., based on the instantaneous firing rate at or near rheobase). As reported previously by Beaulieu-Laroche, L et al (2018), we similarly see a relatively low incidence of bursting among cells from each major layer within our dataset.

156

157   Reviewer #2(Remarks to the Author):

158

159   The authors describe the differences in passive and active membrane properties of L2/3 and L5
160   pyramidal cells in human brain slices obtained mainly from the anterior temporal lobe of
161   epilepsy patients. They found L5 PCs to have higher input resistance, more h-current, and higher
162   gain in response to noisy inputs at delta to theta frequency range compared to L2/3 PCs. The
163   authors propose that these differences in intrinsic properties may serve as the cellular
164   underpinnings for the generation of theta oscillation at deeper layers and its subsequent
165   propagation to more superficial layers, which they (and others) have reported previously.
166   The difference in h-current reported here is not surprising given that it is well established from
167   rodent studies that neocortical L5 PCs, particularly the subcortically-projecting subpopulation,
168   have large h-currents, while L2/3 PCs have little to none (van Aerde & Feldmeyer, 2015). This
169   was also confirmed in human studies conducted from samples similar to what was used in the
170   current manuscript (Kalmbach et al., 2018, Beaulieu-Laroche et al., 2018). Contribution of h-
171   current to increased input impedance at the delta-theta frequency range and intrinsic burst
172   firing has likewise been explored previously in both rodent and human studies (Hu et al., 2002;
173   Kalmbach et al., 2018). A strength of the study lies in its attempt to provide a mechanistic
174   explanation for phenomena that have been observed at a more mesoscopic level in vivo in
175   humans. However, the connection is weak, and most of the findings at the cellular level have
176   already been reported previously from both rodent and human samples. The implied causal
177   relationship between the stronger h-current in human L5 PCs and their increased responsiveness
178   to theta input, despite some existing literature making this argument, is not immediately evident
179   in the current manuscript to be conclusive. In addition, there are serious concerns regarding the
180   electrophysiological data, methodology, and analysis.

181

182   Major points:

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184   1. The overarching theme throughout the manuscript is that the prominence of h-current in
185   human L5 PCs endows them with increased responsiveness to delta to theta frequency inputs as
186   well as intrinsic bursting properties. However, it is unclear that the h-current is responsible for
187   the generation of theta oscillation by influencing the frequency-dependent gain of each cell type,
188   especially considering that the authors report L5 PCs to have higher input resistance and slower
189   membrane time constant compared with L2/3 PCs. It appears that, as a minimum, repeating the
190   frequency-dependent gain analysis in the presence of ZD7288 would be necessary to support
191   the authors' claim in this context.

192   As suggested, we have now repeated the frequency dependent gain analysis experiments in the
193   presence of ZD7288. We found that blocking I_h predominantly abolished the delta peak in L5
194   cells, and to a lesser extent the theta peak in both L2/3 and L5 cells. These data provide further

195 evidence that the I_h in L5 pyramidal cells imbues them with increased responsiveness to delta
196 and theta frequency input.

197 We agree with the reviewer that h-current is but one of many mechanisms for generating theta
198 oscillations, with a number of other candidate mechanisms also possible. We have highlighted a
199 number of these alternative mechanisms in our discussion.

200

201 2. While the criteria are not agreed upon and the terminology is diverse, it is widely accepted
202 that neocortical L5 pyramidal neurons fall largely into at least two major cell types, with
203 distinguishable long-range projection targets, morphology, and membrane properties, including
204 the size of the h-current (Dembrow et al., 2010). The two different subpopulations also exhibit
205 contrasting local and long-range connectivity, at least in different neocortical regions of rodents
206 (Morishima & Kawaguchi, 2006; Morishima et al., 2011; Morishima et al., 2017; Collins et al.,
207 2018; Anastasiades et al., 2018). Additionally, emerging evidence suggests the presence of cell-
208 type specific properties amongst L2/3 neurons specifically in human slices (Kalmbach et al.,
209 2018), specifically that superficial neurons have little to no h-current and deeper neurons have
210 significant amounts. In light of these points, it is unclear why the authors have decided to group
211 all their recordings into L5 vs L2/3. This is a major potential confound for the authors'
212 interpretation that could be addressed with some straightforward classification and/or
213 clustering.

214 We acknowledge this important point that it is extremely likely that there are subtypes within
215 each of the major cortical layer-based groupings that we have presented here.

216 As suggested by this reviewer and reviewer #1, a major limitation with our prior analysis was our
217 somewhat naïve grouping of pyramidal cells into layers. To address this comment, we have
218 conducted a number of additional targeted recordings from Layer 3c, with the goal of confirming
219 the finding that there is a greater degree of sag / h-current in the deeper parts of Layer 3c relative
220 to more superficial cortex.

221 In addition, we now present in Fig 6 an analysis of cell type sub-clusters and gradients based on
222 multi-variate correlations in pyramidal cells' basic electrophysiology features, such as input
223 resistance, resting potential, action potential width at rheobase, etc, with an attempt to connect
224 these gradients to cell morphologies (where available). This analysis suggested that, as described
225 previously, there exists a great degree of heterogeneity within each of the cellular layers
226 sampled here, with cells sampled from Layers 2/3 and 3c often displaying similar
227 electrophysiological features to those from Layer 5 pyramidal cells. Regarding the point about
228 multiple cell types being present, in particular thin- and thick-tufted cells within our data from
229 human L5, we feel that this suspicion is more or less confirmed through a qualitative comparison
230 of our data with the limited reconstructions that were available. However, we note that we were
231 not able to easily parse L5 ET from IT cells from our data with this approach, just that we could
232 likely confirm that such a dichotomy likely is present within our data.

233 This analysis corroborates the reviewer's point and considerable previous work suggesting that
234 there is considerable heterogeneity within cells sampled from each layer (Berg, Sorensen et al.
235 2020), with much of this variability being correlated with morphological differences (such as
236 those shown in (Deitcher, Eyal et al. 2017), and that this comparable to, or even greater than,
237 electrophysiological differences across layers.

238

239 3a. Much of the electrophysiological data presented in the current manuscript are hard to
240 reconcile with previous studies. First, L2/3 PCs were reported to have higher input resistance
241 compared with L5 PCs, in both rodents and in humans (Bitzenhofer et al., 2017; Beaulieu-Laroche
242 et al., 2018; Larkum & Zhu, 2002; Larkum et al., 2007), even though reports in the opposite
243 direction are also present from different authors, albeit not within a single paper (Chang et al.,
244 2005; Luebke et al., 2007). The discrepancy in terms of input resistance may be, however, due
245 simply to technical differences as different groups tend to have different definitions of input
246 resistance and voltage sag in the presence of h-current. It would therefore be helpful if the
247 authors stated this clearly in their methods and discussion the many substantial disagreements
248 with the published literature.

249 We agree that it is surprising that we find that L5 pyramidal cells (as a group) have similar input
250 resistances to L2/3 pyramidal cells (again, as a group). We don't feel that this finding is due to
251 mere differences in how input resistance is calculated, as we obtained similar estimates of input
252 resistances using multiple methods (e.g., manually calculating them in ClampFit, calculating
253 them algorithmically in Python). We acknowledge that it is certainly possible that the input
254 resistances we report here are biased upwards here due to inadvertent cutting of dendrites,
255 especially for larger cells, like those in L5, we have now added a new section to the discussion to
256 further describe this limitation.

257 We contextualize our finding of higher (or similar) group-averaged input resistances between L5
258 and L2/3 pyramidal cells as follows: it is very likely that our dataset is oversampled for thin-tufted
259 compared to thick-tufted cells, especially compared to the L5 pyramidal cells sampled by
260 Beaulieu-Laroche, L et al (2018), which intentionally targeted larger thick-tufted cells, whereas
261 our sampling of cells in L5 tended to be more random. Based on recent single-cell transcriptomics
262 data from human neocortex by Allen Institute that further compared these cells' transcriptomes
263 to orthologous cell types in the mouse, there is strong (transcriptomic) evidence that extra-
264 telencephalic (thick-tufted) cells comprise roughly 1% of the excitatory cells in MTG in human
265 L5, as opposed to approximately 20% in the rodent. Therefore, it is quite likely that most of our
266 (randomly-sampled) cells from L5 are likely to be IT / thin-tufted, and if so, would explain the
267 discrepancy between the relatively large input resistances reported here versus those sampled
268 in Beaulieu-Laroche, L et al (2018) or in previous work in rodents. We note that without
269 additional morphologies or, ideally, molecular corroboration (like with Patch-seq), we cannot
270 conclusively confirm this hypothesis.

271 To further corroborate this point, we note that when we have re-analyzed the publicly available
272 intrinsic electrophysiology data collected from human brain samples from the Allen Institute, we
273 find a similar trend for L5 cells to show larger input resistances than those from L2 and L3 in their
274 cohort and that this trend holds even for cells where the apical dendrite has been annotated to
275 be explicitly intact (Figure S7).

276

277 3b. Second, the gain of the current-frequency relationships in Fig. 3C also appear to be higher
278 compared to previous observations, with L2/3 cells already saturating at +350 pA injection.

279 We thank the reviewer for their comments regarding the current-frequency relationships (FI
280 curves) shown in our prior submission.

281 A detailed reanalysis of our entire dataset (see reviewer comment and response in point 4
282 below) found that some putative fast-spiking interneurons were included in the dataset in our
283 prior submission and thus erroneously included in our prior analysis. These outliers led to the
284 increased gain in the apparent FI curve. In our revised submission (after discarding these putative
285 interneurons), the updated FI curves now correspond much more closely with our expectations
286 and those from the previous literature.

287 We have also included a cursory analysis of the electrophysiological properties of our suspected
288 interneurons in Figure 5.

289 3c. Third, a previous study by Beaulieu-Laroche et al. reported an absence in correlation between
290 patient age and dendritic h-current (albeit with a smaller sample number), which disagrees with
291 one of the major points of the current manuscript. In addition, the correlations the authors
292 report may be subject to methodological and/or statistical problems: despite the massive
293 number of neurons in the Allen Institute dataset for L2/3 neurons, there is essentially no trend,
294 in contrast to the authors' L2/3 data. The authors' L2/3 correlation (Fig. 6C) appears to be
295 strongly driven by a surprising disconnect between the second-oldest patient group in the mid-
296 40s and the oldest patient group of what appears to be early-50s. How is this kind of sampling
297 possible? If the authors left this conspicuous older group out, there would be no correlation
298 across 20 years of patient data. Similarly, the strong correlation in Fig.6A appears driven by a
299 relatively few neurons from 20-25 year olds with low (even negative!) sag ratios, which seems
300 highly implausible. As does the three 80+ year old patients with extremely high sag ratios in the
301 Allen Institute database. These kinds of correlational analyses are fraught with potential
302 confounds, one of which is likely the sampling of different cell types in the different patients.
303 The authors need to do a much more rigorous and principled job of dealing with these data than
304 just blindly fitting scatterplots.

305 We acknowledge each of these very valid concerns with this prior analysis and are grateful for
306 the note that a similar analysis was performed in Beaulieu-Laroche, L et al (2018) with no
307 relationship found between dendritic sag and patient age. The comment that we are likely

322
323   4. The data in the manuscript exhibits a surprising degree of variance, potentially coming from
324   poor experimental quality control and/or inappropriate analyses. For example, Fig. 1 B-D shows
325   enormous ranges for values that are generally agreed upon to be very tightly controlled in a
326   given cell type. A 20 to 30 mV range in resting potential for pyramidal neurons is completely
327   unprecedented and does correspond to the vast majority of previous studies. The same goes for
328   input resistance and membrane time constant. The authors must either explain this remarkable
329   variance or cluster/categorize/curate their data according to previously established criteria.

injection at baseline from these same cells). This correction now addresses some of the concerning large range in RMPs that were raised by this reviewer (Fig 1).

Lastly, we acknowledge that despite this careful curation effort, these data remain quite heterogeneous, especially given what one might expect based on analogous studies in the rodent. Some of this heterogeneity is doubtless due to technical factors given the limitations of the human surgical tissue that are especially challenging to control (e.g., tissue quality). However, as suggested by this reviewer and the other reviewers, some of this heterogeneity is also very likely to be biological, reflecting natural gradients or subclusters within these data (explored in Fig 6 and discussed in detail in the Discussion).

5. References are placed in inappropriate contexts. As an example, the authors reference four papers by Sakmann and Petersen groups in support of their statement that L5 PCs are more excitable than L2/3 cells (page 20). However, all of these papers are exclusively about L2/3 PCs, and none of them mention L5 PCs (except a couple of deeper-positioned cells in the supplement for one of the papers), let alone their input resistances. Furthermore, all of these experiments were conducted in vivo, wherein synaptic conductances will reduce the apparent input resistance of the cell (Destexhe et al., 2001), making direct comparisons with in vitro studies from slices inappropriate. As another example, the authors point to Larsen et al. (2008) to describe the connection properties of intrinsically bursting cells, but said paper is strictly structural and does not discuss electrophysiology. This issue needs to be addressed.

Thank you for pointing this out. We have now removed these erroneous citations in the revision.

6. The authors state that they used paired or unpaired t-tests (page 7), except for frequency-dependent gain analysis. The majority of the data, however, are obviously not in normal distribution. Nonparametric tests would therefore be more appropriate.

We have replaced our previous statistical tests with non-parametric Wilcoxon tests.

7. Whole-cell voltage-clamp is not quantitative in neurons with dendrites and voltage-dependent ion channels. It is not clear what the data in G-I add to the manuscript. It should be removed.

We appreciate the reviewer's comment regarding the well-known space clamp issues that limit our ability to adequately clamp these cells' voltage. We acknowledge that these issues would be especially pronounced for human pyramidal cells given their large size.

Despite these known limitations, we thought that there would be some value in attempting to use voltage-clamp to help provide kinetics and voltage dependency characteristics for the human I_h. In light of the concerns raised by the reviewer, we have now elected to 1) move these data to the supplemental figures (Figure S2); and 2) to address these space clamp issues in the manuscript text to allow the reader to better contextualize our findings given these limitations.

382

383   Minor points:

384

385   1. The depths of the cell bodies for the reconstructed cells in Fig. 1A don't correspond to the

386   anatomical images: are L2/3 and L5 PCs offset from one another, or on different scales? The

387   authors should show laminar borders for these reconstructions if that is how they define L2/3 vs

388   L5. A further anatomical point: are the authors concerned that what they are calling L5 PCs

389   appear to have no apical tuft dendrites?

390   We have now updated the presentation of the cell morphologies in Fig1A to address this point

391   of clarity.

392

393   2. The authors describe that "the half-width of the action potential was shorter in L5

394   pyramidal cells compared to L2/3 pyramidal cells" (page 13). The associated p-value,

395   however, is 0.07 and should be considered nonsignificant. Either correct the p-value or

396   rephrase the statement.

397   Corrected in the text.

398

399   3. From the solution compositions in methods, it would be more likely for the liquid junction

400   potential to be - 10.8 mV, instead of the + 10.8 mV as appears on the manuscript (page 4).

401   Corrected in the text.

402

403   4. "The membrane time constant (tau_m) was calculated using a single-exponential fit of the

404   membrane potential response to a small hyperpolarizing pulse." (page 4): what was the

405   amplitude of the hyperpolarizing current injection (instead of "small")?

406   The methodology for calculating membrane time constant has now been more explicitly

407   described.

408

409   5. "I_h appeared to be one of the major contributors to the prominent post-inhibitory

410   depolarization" (page 20): Hyperpolarizing current injection is not "inhibitory" per se.

411   Corrected in the text.

412

413   6. The definitions of stimulus-response correlation (Csr) and stimulus-stimulus autocorrelation

414   (Css) are not given in the methods (page 6). They are given in the referenced Higgs & Spain paper.

415   We now include the definitions of these quantities.

416

417   7. Referring to the resonance frequency in the impedance amplitude profile formed by h-current

418 as "low frequency membrane resonance" (page 15) sounds misleading because the effects of
419 the h-current is to impose a high-pass filter. Perhaps rephrasing this could be better.

420 Corrected in the text.

421

422 8. There are numerous typographical errors throughout the manuscript that pose as distractions
423 for the reader. Some, but not all, examples include: Repeated occurrences of "*, **,***, indicate
424 significantly different at P < 0.001 , P < 0.05, P < 0.001" (in legends), "L5: 68 ± 8mV" (page 9,
425 describing RMP), "lager sag" (page 10), "we also we performed" (page 11), "we surprisingly we
426 found" (page 15), "Wilcox test" (page 18), "I_Nap" (page 20, with undercase p), "Hu et. al" (page
427 21), and initials of the first author (page 22).

428 Corrected in the text

429

430

431 Reviewer #3 (Remarks to the Author):

432

433 This is a thorough, careful and thoughtful MS quantifying the distribution of Ih in human – mainly
434 anterior temporal – neocortex by layer. I completely concur with the ethos behind the study –
435 that modelling the human brain demands constraint by human data and, for this reason alone,
436 the paper constitutes a valuable reference work and so to me, deserves publication.
437 I do have a couple of issues with the MS as it stands though. The comments below are, hopefully,
438 easily dealt with and are intended merely as suggestions to improve the MS.

439

440 1) I see why the authors 'hang' their findings on the human theta rhythm. But I don't think its
441 valid given precedents in rodent literature. As they argue in the discussion, there are multiple
442 mechanisms that can underlie neuronal rhythmicity at theta frequency. Ih is almost certainly not
443 one of them in neocortex. Hippocampal theta cannot reliably be considered the same
444 phenomenon, the frequencies are different for a start. Here there is a dependence on Ih but it
445 lies principally in the behaviour of a subset of interneurons and the neocortical equivalents were
446 not examined in the present MS. In addition, Ih is exquisitely dependent on neuromodulatory
447 state: Theta is mainly seen in non-invasive human recordings in the wake state but, in this
448 condition, multiple wake-associated neuromodulators all act to reduce Ih considerably (e.g. Ach,
449 Orexin/hypocretin etc.). What Ih IS critically involved in is rebound following synaptic inhibition.
450 Thus it plays a crucial role in delayed
451 responses to sensory input (the 'off' response) and 'anodal break' spiking seen in mismatch
452 responses.

453 We thank the reviewer for this thorough and positive comment. We indeed agree with the
454 reviewer's analysis that theta rhythmicity is a complex dynamic, both at the cellular and network
455 level, that is driven by a wide variety of factors beyond I_h, and that there are important

456 differences between human and rodent theta rhythms. We have thus endeavored to downplay
457 the emphasis on theta resonance. Our ZD-7288 (I_h blocker) experiments firmly establish that the
458 low frequency peaks in G(f) particularly in the delta frequency are dependent on I_h. In light of
459 these new results, we interpret our previous findings of interlaminar coherence at theta
460 frequency (4-8Hz), as arising from the dynamic interaction between IB cells (that burst at delta),
461 and RS cells (which are the predominant cell-type we likely recorded from) that receive delta
462 frequency input from IB cells to which they are particularly tuned to (peak in delay in G(f)), which
463 then discharge at theta frequency (and possibly in part the theta frequency peak in G(f)). Please
464 see response to last comment for a continuation of this discussion.

465

466 2) The data is presented with commendable clarity, but consequently suggests multimodal
467 distributions in a number of the intrinsic cell properties measured. This is discussed briefly (L2 vs
468 L3 for example) but the main subdivision of cell type I know of that manifests in part as sag
469 amplitude differences is between L5a and L5b. The authors have gathered an impressive set of
470 data so I wonder if it is possible to stratify L2/3 and L5 cell types further on the basis of other
471 intrinsic properties (slow AHP, burst generation, afterdepolarisation strength etc.) This may help
472 to 'clean up' the often very broad distributions in some of the metrics.

473 We thank the reviewer for this excellent comment. We point the reviewer to our analysis in (Fig
474 6), which attempts to address some of the large degree in heterogeneity within cells from the
475 same cortical layer.

476 We also note that we address this topic in our responses to similar comments from the other
477 reviewers: specifically, we have addressed some of this increased variability by identifying
478 interneurons and distinct experimental protocols that were mistakenly included in our data set,
479 while noting that some level of increased heterogeneity in these cells (relative to the rodent
480 setting) is to be expected based on the emerging human cortical cell typing literature.

481

482 2 minor points:
483 3) Lack of observed resonance on somatic recordings with patch electrodes is not surprising.
484 Patch solutions dialyse cytosol hugely and thus interfere with many intrinsic conductances. In
485 addition, the distribution of Ih in neurons is not uniform and resonance can be seen in dendritic
486 recordings in a given cell type when it appears completely absent in somatic recording.

487 We thank the reviewer for noting these potential confounds in our subthreshold resonance data.
488 We have now added a paragraph to our discussion within our limitations section to contextualize
489 how experimental confounds, such as dendrite cutting or cytosol dialysis, might influence some
490 of our ability to observe subthreshold resonance in these data.

491 4) The MS data does agree with the Carracedo data in terms of layers showing most theta
492 (discussion). In that paper the field theta was mainly manifest in superficial layers but the origin

493 of the synaptic activity underlying this was exclusively intrinsic theta activity in a subpopulation
494 of L5 cells. See point 1 above though, this theta was not Ih-dependent.

495 We thank reviewer for this excellent comment and insights. We fully agree that I_h is likely not
496 directly responsible for the theta frequency activity in local circuitry. We do however argue in
497 line with the Carracedo, L et al (2013) work, that it is indirectly complicit in theta generation
498 given our new experiments added to this MS, that show that the delta peak (~2Hz) in *G(f)*, and
499 less so the theta peak (~7Hz) are dependent on I_h (see figure 7E-F). From the Carracedo, L et al
500 (2013) paper it was the RS cells that generated theta, likely the cell-type that primarily
501 contributed to *G(f)* (Figure 7B). We speculate that they did not observe theta in the deep layers,
502 since human circuits appear to amplify local activity relative to rodent cortex (Molnár, Oláh et
503 al. 2008) which might make theta more prominent human L5. Conversely, we did not observe
504 delta activity in our previous in-vitro recordings, possibly due to the paucity of IB cells in human
505 middle temporal gyrus cortex (Hodge, Bakken et al. 2019). Our data as well provides a putative
506 cellular mechanism (peaks in *G(f)*) underlying these population activities. We have schematized
507 these ideas below and include it as a supplementary (Figure S8) and clarified this in the
508 discussion.

509
510
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514
515
516
517
518
519

Hz
2   7

IB   RS   I   RS

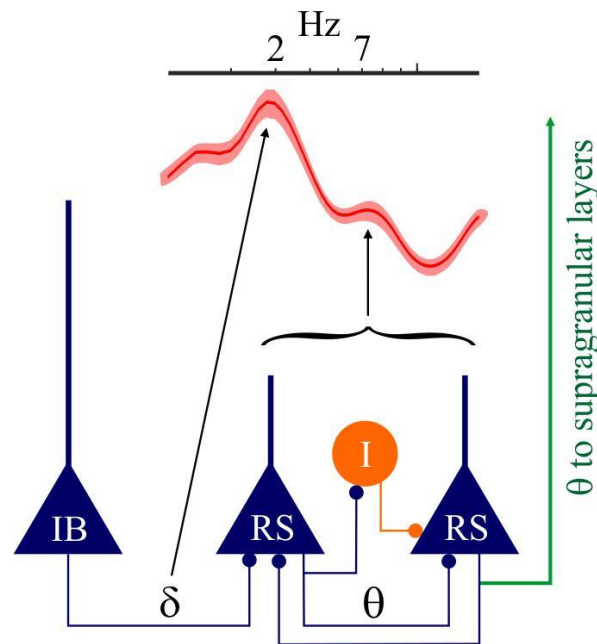θ to supragranular layers

δ   θ

Figure S8: Structural circuit motif for L5 theta oscillations. Delta frequency output from intrinsically
bursting (IB) (Carracedo, Kjeldsen et al. 2013) neurons is well tracked by regular spiking (RS) cells that
have a peak in *G(f)* (red) (figure 7B in main text) at delta. In turn RS cells that are poorly adapting, have
steep f-I curves, and low rheobase discharge at theta frequency (Carracedo, Kjeldsen et al. 2013). RS cell
drive local circuits at theta including other RS cells that track theta well via the peak at 7Hz in G(f).
Interneurons amplify local activity through rebound excitation (I; orange; see Figure 5 main text) in
human circuits that are predisposed to reverberant activity (Molnár, Oláh et al. 2008).

527

528

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547

Reviewer #1 (Remarks to the Author):

The authors have done a nice job of being responsive and open to the reviewer concerns and addressing the major issues raised. I appreciate the willingness of the authors to acknowledge various flaws that were either directly called out or discovered on their own in the course of revision. The corrections and new experimental data collected were quite important and no doubt have strengthened the manuscript over the original submission. I acknowledge that quite a lot of new experimental and analysis work was completed for this revision. Given the substantial new material introduced, I have additional comments on those changes, and these new comments are intended to help further strengthen the manuscript. I believe the manuscript could be acceptable for publication in Nature Communications pending these additional relatively minor changes.

Conducting additional experiments on L3c pyramidal neurons was a reasonable compromise to get at gradients of features for supragranular pyramidal neuron population. This was an acceptable choice given that targeting of rare L5 thick tufted neurons in the human cortex would be exceptionally challenging. The conclusions are limited by the lack of L5 ET neurons in the data set, but this caveat is reasonably well discussed and acknowledged), and I find that acceptable.

Regarding L2/3 vs L3c, I worry that this naming scheme may cause confusion for readers familiar with rodent work on L2/3, as there are in fact discrete layers 2 and 3 anatomically in the human cortex. The authors should consider whether this can be better solved in the presentation of the data.

Line 483-484 should be corrected for grammar, e.g. "These results suggest that depth from pia appears to be a general organizing principle for sag current in the cortex for both excitatory and inhibitory neurons." I'm not so sure organizing principle is the correct way to pitch this observation. It is an interesting observation, and is the only real justification to keep this as a main figure. It is a little odd to include this where interneurons in L2/3 and L5 are called "putative" and no information on the diversity of interneuron types is readily available for these data, especially given that the rest of the paper is focused on excitatory neurons across layer. To me this seems to be a supplemental figure at best, and still feels preliminary. The principle result of sag currents being larger in deeper layers relative to upper layers may be a real phenomenon, or it may be a result of interneuron subtype sampling bias in this small interneuron dataset. The authors should consider this issue and at least acknowledge the limitations more directly in the text.

Regarding Figure S4, are these two neuron morphologies shown at matched scale? Likely they are not. It is a peculiar non-pyramidal neuron morphology indeed.

Figure S7: It is striking that the input resistance ranges are completed overlapping between Krembil and Allen data for L2 and L3 pyr neurons, yet the distributions are nearly non overlapping for L5 neurons, which can't be fully accounted for by truncation status. I imagine this discrepancy can't be resolved in the present study, yet it is notable. Despite this fact, it is commendable that the authors utilized this publicly available data set to compare to their data set and attempt to corroborate several findings.

The novel visualization in Fig 6 is interesting but provides more questions than discrete answers. The recent transcriptomics data strongly suggests that layer membership is not particularly informative regarding cell type diversity in human cortex, as many discrete cell types to not obey strict laminar

boundaries. It might be worth expounding a little more on the point that layer is not a good surrogate for discrete cell types.

Reviewer #2 (Remarks to the Author):

For this revised manuscript, the authors report five main findings from their study on human neocortical pyramidal cells: 1) a gradient of increasing intrinsic excitability and Ih from superficial to deeper layers; 2) Ih -dependent enhancement of neuronal gain at delta and theta frequencies for layer 5 cells; 3) subthreshold resonance found in pyramidal cells; 4) heterogeneity in membrane properties; and 5) a possible correlation of Ih with patient age.
Most of these results are not new, having been previously investigated quite deeply, as was mentioned in the preceding round of review. The contribution of Ih to membrane resonance and its potential link with neural oscillation has been known for decades (Hutcheon et al., 1996). The positive correlation between Ih and somatic depth of human layer 2/3 pyramidal cells as well its contribution to frequency-dependent power were also recently described in detail (Kalmbach et al., 2018). Stronger Ih in thick tufted human layer 5 pyramidal cells compared with layer 2/3, consistent with many previous observations from rodents, has likewise been documented (Beaulieu-Laroche et al., 2018). A large proportion of remaining data in the current manuscript, such as action potential waveform analysis, is potentially interesting, but seems detached from the main direction of the study.
The authors emphasize two findings in particular as important: direct experimental demonstration of the consequence of Ih block on frequency-dependent gain in layer 2/3 and 5 pyramidal cells (which was suggested in the previous round of review) and the potential correlation between Ih and age. However, these results are both not surprising based on previous knowledge and inadequately supported by the evidence provided. Additionally, serious concerns persist regarding methodology, data curation, and interpretation; this accrues with inconsistencies between the current study and the existing literature, calling in to question what descriptive value remains.

Major points:
1. The large majority of cells are shown to be non-resonant regardless of their position, with fewer cells displaying very low resonance frequency typically around 1 Hz even though representative examples are taken from the extremes (Fig. 4C). In contrast, Kalmbach et al. report the majority of layer 2/3 cells to be at least modestly resonant, especially in but not limited to the deepest layer 3 cells (Fig. 4B & D of Kalmbach et al.), along with considerably higher resonance frequency compared with values in the current manuscript. While the possible cause of inconsistency between these data is not discussed, the authors address this lack of subthreshold resonance by noting that there are other known contributors to membrane resonance. Unlike Kalmbach et al., such small fraction of resonant cells with very low resonance frequencies do not appear to be in good alignment with, or at least immediately relevant to, one of the central arguments of the manuscript, that Ih shapes neuronal resonance properties in such way that results in enhanced input-output relationship at delta-theta range for layer 5 cells (Fig. 7F). It could be argued that the difference may originate from the subthreshold vs. suprathreshold nature of the stimulation protocols used; even so, the frequency-dependent gain profile from noisy current input (Fig. 7B) is not consistent with that from sinusoidal current input either (Fig. 4F), where the significant and roughly twofold difference in spike probability at higher frequencies as shown in the latter disappears from the former. More apparent discrepancies can be found from Fig. 7, wherein the frequency-dependent gain profiles in panels B vs. E & F show striking difference in tendency which in some ways even appear to be reversed,

particularly for layer 5 cells. It is surprising in this regard that, despite such large variances in data, that the authors felt showing n = 3 cells without any statistical analysis was appropriate. I don't even see any error bars. I do not find this experiment convincing, particularly because it's not clear how these few cells were chosen from the heterogenous population shown in Fig 4.

2. It is remarkable that such cells as cell f or cell g shown in Fig. 1, which only reach up to layer 3 or even the border between layer 3 and 4, are presented as representative examples of layer 5 pyramidal cells that are presumed to be intact along the longitudinal axis. Cell g is further proposed to be a thick tufted layer 5 pyramidal cell (line 539), even though its morphological reconstruction simply lacks a tuft. Such features are completely unencountered in rodents and, to our knowledge, in humans as well. Note that the spiny neurons that are labeled as intact in the Allen Institute database whose electrophysiology and morphology are available (of which there are only two cells) also have visible apical dendrites reaching all the way up to layer 1, while at the same time displaying classic morphological features of neocortical layer 5 pyramidal cells. In addition, it is worthwhile to note here that the presence of spines alone is not a guarantee for a cell to be pyramidal or even excitatory, and interneurons including the sparsely spiny interneurons are also found more frequently in primates than in rodents (Kawaguchi & Kubota, 1993; DeFelipe, 2011). Whether the apical dendrites of cells recorded are intact is of much importance for the current study, not only as it will affect passive membrane properties but more importantly because ion channels including HCN channels are known to be expressed in steep gradients along the somatodendritic axis (Lorincz et al., 2002). It is also unclear from the manuscript how the authors excluded the non-fast-spiking interneurons (which coincidentally have high input resistance) from analysis, which are much less obviously distinguishable from pyramidal cells by electrophysiology alone to the untrained eye. The authors describe that putative interneurons were identified by spike properties such as action potential width, maximum firing rate, and strong afterhyperpolarization, all of which are well-suited criteria for fast-spiking interneurons such as those shown as representative examples, but much less effective for identifying non-fast-spiking interneurons (a population which also overlaps with the aforementioned sparsely spiny interneurons such as calbindin-expressing interneurons in the deeper layers of the neocortex).

3. The authors continue to place irrelevant and inappropriate references in attempts to support their otherwise unfounded claims, even after having been corrected for numerous such instances from their previous manuscript. For example, they state that "human neocortical circuits demonstrate […] different short-term plasticity rules, compared to neocortical circuits in rodents"; setting aside the ambiguity on exactly what type of synapses the authors are referring to here, one of the two cited papers is entirely on rhythmic firing in rat layer 5 pyramidal cells, while the other is about timing rules for spike timing dependent plasticity in human hippocampal (not neocortical) neurons. Neither study addresses or even mentions short-term plasticity. Another example is their statement that "Ih contribute[s] to low pass filtering properties of pyramidal cells", which first of all may not be technically correct under normal conditions; second, seems to contradict the authors' own data (Fig. 7F; S2A); and third, inappropriately references a study that certainly does not make any such claim.

4. It is disturbing that the authors were able to find and remove significant parts of their original data that were supposedly included in error but went unnoticed, sometimes resulting in revoking their original conclusions (i.e. strong correlation of Ih and patient age). Taking this into account with the other inconsistencies and methodological concerns that are still present, it is challenging to come away from this paper with any strong insight about human neurons and how their intrinsic properties may contribute to brain level oscillations.

5. The unexplained variance in basic physiological properties continues to be a subject of concern. The RMP for L2/3 neurons spans from -60 to -80 mV; for L5 it's -80 to -55 mV. The authors acknowledge this may be either biological variation or an experimental confound, but do nothing further to try to resolve this. If the authors performed a set of similar experiments on mouse or rat cortical neurons in L2/3 or L5 under the conventional, highly stereotyped conditions of brain slice preparation, would they still see this level of variance? How much of the variance in this manuscript is experimenter-driven quality control in terms of slice preparation and patch-clamp prowess and how much is a result of other forces, like real biological variability and/or human brain tissue condition? Speaking of which, are there inclusion criteria for human brain samples? One can imagine that depending on specifics of the surgery and the patient, the tissue could be in very different states, potentially contributing to the health on individual neurons and their resting membrane potential. How do the authors deal with this?

In summary, is it not clear what the authors want the reader to conclude at the end of this manuscript. The experiments and analysis have all largely already been performed in human neurons, and the attempt to connect these cellular properties to oscillatory dynamics, as has already been done in rodents, is weak. It is thus hard to understand how the current work will have a substantial impact in the field.

Reviewers' comments

Reviewer #1:

The authors have done a nice job of being responsive and open to the reviewer concerns and addressing the major issues raised. I appreciate the willingness of the authors to acknowledge various flaws that were either directly called out or discovered on their own in the course of revision. The corrections and new experimental data collected were quite important and no doubt have strengthened the manuscript over the original submission. I acknowledge that quite a lot of new experimental and analysis work was completed for this revision. Given the substantial new material introduced, I have additional comments on those changes, and these new comments are intended to help further strengthen the manuscript. I believe the manuscript could be acceptable for publication in Nature Communications pending these additional relatively minor changes.

We thank the reviewer for their positive comments on our work.

Conducting additional experiments on L3c pyramidal neurons was a reasonable compromise to get at gradients of features for supragranular pyramidal neuron population. This was an acceptable choice given that targeting of rare L5 thick tufted neurons in the human cortex would be exceptionally challenging. The conclusions are limited by the lack of L5 ET neurons in the data set, but this caveat is reasonably well discussed and acknowledged, and I find that acceptable.

We again thank the reviewer for their positive comments and for recognizing the additional experiments involved in our first round of revisions. In addition, Reviewer #1 has recognized one of the key messages of this manuscript: there is a clear diversity amongst pyramidal cells in human cortical L5, and we have situated our conclusions within this context.

Regarding L2/3 vs L3c, I worry that this naming scheme may cause confusion for readers familiar with rodent work on L2/3, as there are in fact discrete layers 2 and 3 anatomically in the human cortex. The authors should consider whether this can be better solved in the presentation of the data.

We thank the reviewer for this insightful point. In our revised manuscript, we have changed the naming scheme from L2/3 to L2&3, and discussed this rationale in the Methods **(Lines 135-137).** We feel this subtle change highlights that L2 and L3 are distinct in the human cortex (rather than indistinguishable in the rodent cortex), while not making this new naming schema unnecessarily confusing for those coming from the rodent setting.

Line 483-484 should be corrected for grammar, e.g. "These results suggest that depth from pia appears to be a general organizing principle for sag current in the cortex for both excitatory and inhibitory neurons." I'm not so sure organizing principle is the correct way to pitch this observation. It is an interesting observation, and is the only real justification to keep this as a main figure. It is a little odd to include this where interneurons in L2/3 and L5 are called "putative" and no information on the diversity of interneuron types is readily available for these data, especially

given that the rest of the paper is focused on excitatory neurons across layer. To me this seems to be a supplemental figure at best, and still feels preliminary. The principle result of sag currents being larger in deeper layers relative to upper layers may be a real phenomenon, or it may be a result of interneuron subtype sampling bias in this small interneuron dataset. The authors should consider this issue and at least acknowledge the limitations more directly in the text.

We very much appreciate this comment on one of the new figures in our manuscript. In our revised manuscript, we have adjusted the text **(Lines 584-597)** to more conspicuously acknowledge the limitations of this preliminary exploration of putative interneurons reported here. We have also taken the reviewer's suggestion to make this a supplementary figure **(Fig. S7).**

Regarding Figure S4 (note from the authors: this is now Figure S8 in the resubmitted manuscript), are these two neuron morphologies shown at matched scale? Likely they are not. It is a peculiar non-pyramidal neuron morphology indeed.

The scale was indeed correct for these two neurons. However, the two neurons were previously imaged in different fashions, due to COVID-19 restrictions that prevented a full 3D reconstruction of the non-pyramidal cell, and we used what was available to us to reconstruct a 2D morphology. With these restrictions loosened over the past few months, we have been able to perform a proper 3D reconstruction of this non-pyramidal neuron that should resolve any concerns in this regard (now **Figure S8**).

Figure S7 (note from the authors: this is now Figure S1 in the resubmitted manuscript): It is striking that the input resistance ranges are completed overlapping between Krembil and Allen data for L2 and L3 pyr neurons, yet the distributions are nearly non overlapping for L5 neurons, which can't be fully accounted for by truncation status. I imagine this discrepancy can't be resolved in the present study, yet it is notable. Despite this fact, it is commendable that the authors utilized this publicly available data set to compare to their data set and attempt to corroborate several findings.

We thank this reviewer for acknowledging our sincere attempts to reconcile our data with analogous datasets from the Allen Institute and elsewhere.

We also were puzzled that the input resistance values from the Allen Institute public L5 pyramidal cells were even larger than those reported within our primary dataset. However, both ours and the Allen datasets showed considerably larger input resistances than the thick-tufted L5 cells reported in Beaulieu-Laroche et al. (Beaulieu-Laroche, Toloza et al. 2018), further suggesting that ours and the Allen L5 data are perhaps more likely to be IT versus ET projecting cells. However, further reconciling such differences, such as input resistance values, between each of these datasets will likely require more robust approaches for identifying the same cell type across datasets, such as Patch-seq or the use of viral tools.

The novel visualization in Fig 6 is interesting but provides more questions than discrete answers. The recent transcriptomics data strongly suggests that layer membership is not particularly

informative regarding cell type diversity in human cortex, as many discrete cell types to not obey strict laminar boundaries. It might be worth expounding a little more on the point that layer is not a good surrogate for discrete cell types.

We thank the reviewer for this observation. We agree that one of the major points of this analysis is that it further highlights that layer membership is not a particularly informative dimension, on its own, for cell grouping (a point that also corroborates recent transcriptomic evidence). In our revision we have discussed this specific point in new text **(Lines 567-570)** as well as in a paragraph in the Discussion beginning on **Line 625.**

Reviewer #2:

For this revised manuscript, the authors report five main findings from their study on human neocortical pyramidal cells: 1) a gradient of increasing intrinsic excitability and Ih from superficial to deeper layers; 2) Ih -dependent enhancement of neuronal gain at delta and theta frequencies for layer 5 cells; 3) subthreshold resonance found in pyramidal cells; 4) heterogeneity in membrane properties; and 5) a possible correlation of Ih with patient age.

Most of these results are not new, having been previously investigated quite deeply, as was mentioned in the preceding round of review. The contribution of Ih to membrane resonance and its potential link with neural oscillation has been known for decades (Hutcheon et al., 1996). The positive correlation between Ih and somatic depth of human layer 2/3 pyramidal cells as well its contribution to frequency-dependent power were also recently described in detail (Kalmbach et al., 2018). Stronger Ih in thick tufted human layer 5 pyramidal cells compared with layer 2/3, consistent with many previous observations from rodents, has likewise been documented (Beaulieu-Laroche et al., 2018). A large proportion of remaining data in the current manuscript, such as action potential waveform analysis, is potentially interesting, but seems detached from the main direction of the study.

The authors emphasize two findings in particular as important: direct experimental demonstration of the consequence of Ih block on frequency-dependent gain in layer 2/3 and 5 pyramidal cells (which was suggested in the previous round of review) and the potential correlation between Ih and age. However, these results are both not surprising based on previous knowledge and inadequately supported by the evidence provided. Additionally, serious concerns persist regarding methodology, data curation, and interpretation; this accrues with inconsistencies between the current study and the existing literature, calling in to question what descriptive value remains.

We appreciate the detailed reading of our manuscript that yielded "five main findings" as assessed by the reviewer. However, we feel that this contextualization of our manuscript minimizes two of the most important findings of our manuscript: first, that there is immense diversity amongst pyramidal cells in human L5, and second, that the correspondence between a large sag current (and corresponding activity of the h-current) and subthreshold resonance is not nearly as direct

as has been suggested by previous studies, including those cited by the reviewer here. These minimizations appear to arise from oversights regarding the literature on the electrophysiological characterization of human neurons and the study of resonance, which we will detail in the following.

Additionally, upon re-reading the manuscript with these comments from Reviewer #2 in mind, we recognized that our title and abstract might have contributed to a misplaced emphasis in the importance of our main findings. Therefore, we have **reworked the abstract** in this most recent revision, as well as **retitled the manuscript** as "Diversity amongst human cortical pyramidal neurons revealed via their sag currents and frequency preferences."

We have also **eliminated the discussion of the correlation between I_h and patient age from the manuscript** entirely to avoid this "side-argument" confounding the larger results put forth in this paper.

Finally, in our revised manuscript we have **explained our findings in light of those from other groups**, including a more in-depth comparison of subthreshold resonance, morphologies, and intrinsic features such as resting potentials. In addition, we have more thoroughly compared our physiological and morphological findings of L5 human pyramidal cells to those of other groups and utilized results from other methodologies such as single-nucleus transcriptomics.

Major points:
1. The large majority of cells are shown to be non-resonant regardless of their position, with fewer cells displaying very low resonance frequency typically around 1 Hz even though representative examples are taken from the extremes (Fig. 4C). In contrast, Kalmbach et al. report the majority of layer 2/3 cells to be at least modestly resonant, especially in but not limited to the deepest layer 3 cells (Fig. 4B & D of Kalmbach et al.), along with considerably higher resonance frequency compared with values in the current manuscript. While the possible cause of inconsistency between these data is not discussed, the authors address this lack of subthreshold resonance by noting that there are other known contributors to membrane resonance.

We agree with the reviewer that the overall fractions of resonant cells as well as their resonant frequencies in our analysis are lower than those reported in Kalmbach et al. (Kalmbach, Buchin et al. 2018). However, our data replicates the trend reported in Kalmbach et al. that there is enhanced resonance for deeper layer L3 relative to more superficial cortical layers.

In this light we now more clearly explain and contextualize our results regarding subthreshold resonance with those reported in Kalmbach et al. (Kalmbach, Buchin et al. 2018). on **Lines 460 to 468.** This includes a direct discussion of the experimental limitations impacting the observation of subthreshold resonance on **Lines 465-468**. These limitations include the possibility of inadvertent dendrite truncation which might affect the observation of subthreshold resonance. An additional explanation is that such differences might arise due to differences in slice preparation and external and internal solutions used during recordings (e.g., we did not use synaptic blockers in our ZAP recordings).

Lastly, a likely alternative explanation consistent with our recent modeling work (Rich, Moradi Chameh et al. 2020) is that we delivered ZAP current stimuli at our cells' RMP, which tended to be slightly more depolarized than those in Kalmbach et al. (which we suspect to arise from solution differences, like those referred to above). This RMP difference likely influences the observation of resonance, including the fact that we are less likely to observe resonance than in the relevant figures in Kalmbach et al. referred to by the reviewer. This theory is supported by a relevant supplemental figure from Kalmbach et al. illustrating that when ZAP is delivered at a -65 mV holding potential (near the RMP for our L2&3), numerous cells were not resonant (i.e., they displayed a resonant peak at or near 0Hz, figure reproduced below). We note that the finding that subthreshold resonance at higher frequencies is more likely to arise at more hyperpolarized voltages, a conclusion drawn from the synthesis of the results of Kalmbach et al. and this study, comports with predictions made in our recently published modeling work (Rich, Moradi Chameh et al. 2020).
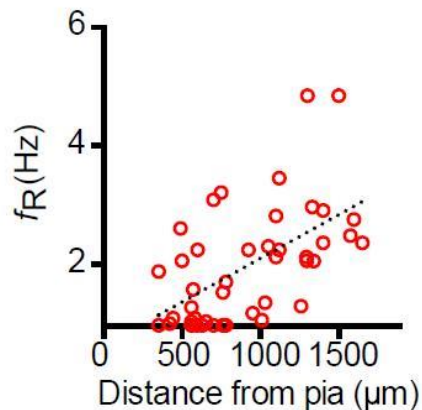
Quoting from **Lines 460-468**: "*These results are generally consistent with recent evidence for greater subthreshold resonance in the deeper part of the supragranular layers of the human neocortex relative to more superficial neurons (Kalmbach, Buchin et al. 2018). While we observed a smaller fraction of resonant cells than previous work, we note that our results correspond with the conclusion that human L2&3 pyramidal cells are most likely to have normalized impedance peaks at <2 Hz, while neurons with peaks at >4 Hz are quite rare. Possible explanations for the lower fraction of resonant cells in our data include our use of different experimental solutions than Kalmbach et al., as well as the possibility of inadvertent dendrite truncation in these experiments (see Discussion). Additionally, our neurons displayed a slightly more depolarized RMP (Kalmbach, Buchin et al. 2018), which is a determinant of observing resonance.*"

Unlike Kalmbach et al., such small fraction of resonant cells with very low resonance frequencies do not appear to be in good alignment with, or at least immediately relevant to, one of the central arguments of the manuscript, that Ih shapes neuronal resonance properties in such way that results in enhanced input-output relationship at delta-theta range for layer 5 cells (Fig. 7F) (note from the authors: this is now Figure 5f in the resubmitted manuscript),. It could be argued that the difference may originate from the subthreshold vs. suprathreshold nature of the stimulation

protocols used; even so, the frequency-dependent gain profile from noisy current input (Fig. 7B) (note from the authors: this is now Figure 5b in the resubmitted manuscript), is not consistent with that from sinusoidal current input either (Fig. 4F), where the significant and roughly twofold difference in spike probability at higher frequencies as shown in the latter disappears from the former. More apparent discrepancies can be found from Fig. 7 (note from the authors: this is now Figure 5 in the resubmitted manuscript),, wherein the frequency-dependent gain profiles in panels B vs. E & F show striking difference in tendency which in some ways even appear to be reversed, particularly for layer 5 cells.

In the revised manuscript, we have added text in multiple locations to better explain the perceived inconsistencies between our subthreshold resonance, suprathreshold resonance, and frequency dependent gain results. We feel that the reviewer's comment potentially represents a misconception regarding the relationship between these three distinct measures and I_h. We will expand upon this issue in more detail than allowed by the length limitations of the manuscript below.

It is first worth clarifying that we make no claim that "I_h shapes neural **resonance** properties in such way that results in enhanced input-output relationship at delta-theta range for layer 5 cells" (emphasis added). It remains an open question whether there is a "one-to-one" relationship between subthreshold resonance and suprathreshold frequency preference, as implied by this comment: in fact, computational and mathematical research (see new citations and text at **Lines 473-475** has directly shown that the impact of subthreshold frequency preference on suprathreshold frequency preference is at best unclear. Our recent modelling work (Rich, Moradi Chameh et al. 2020) also supports the notion that resonance is a complex interplay between intrinsic membrane conductances, passive membrane properties, and other neural features, that makes this story more complex. We feel this point may have been further confounded by a misunderstanding by the reviewer regarding frequency dependent gain and suprathreshold resonance: namely, frequency dependent gain is a *distinct* measure from either sub- or suprathreshold resonance. While the analysis of a suprathreshold ZAP current via impedance plots measures the likelihood of a spike occurring from a sinusoidal input at a specific frequency, the frequency dependent gain measures the likelihood of a neuron spiking in phase with an oscillatory input that is relatively small (Yu and Lewis 1989, Higgs and Spain 2009). *These two measures provide complimentary, rather than superimposable, findings.*

We hope that this explanation helps to clarify why we feel that these points brought up by the reviewer do not represent inconsistencies. In an endeavor to make these nuances clearer to the reader, we have made the following changes to the manuscript:
  ● A revised statement of our main hypothesis on **Lines 75-77** clarifying that we propose that different biophysical and active properties (including, but not limited to, I_h) between superficial and deep layer human neurons underlie the dominant role of deep layer human neurons in driving interlaminar coherence: " *Based on our previous findings that deep layer activity appears to drive superficial activity in the human cortex (Florez, McGinn et al. 2015), we hypothesized that this "leading" role in generating interlaminar coherence*

can be attributed in part to the differing intrinsic properties of deep layer from superficial layer neurons."

- An explicit mention of the distinct nature of frequency dependent gain analysis relative to resonance analysis in the Methods at **Lines 225-227**: "*This measure identifies the likelihood of the neuron spiking in phase with an oscillatory input that is small relative to the overall input to the cell, distinct from analysis of the neuron's activity in response to a suprathreshold ZAP input (Higgs and Spain 2009).*"

- A more detailed comparison between the conclusions that can be drawn from both resonance and frequency dependent gain analysis, and their distinctness, before the presentation of the frequency dependent gain results in the Results at Lines **497-502:** "*This measure captures distinct neuronal features compared to sub- or suprathreshold resonance: while resonance identifies the likelihood of a spike occurring from a drive at a particular frequency that is itself is suprathreshold, the frequency dependent gain quantifies the phase preference of neuronal spiking as a function of frequency (Yu and Lewis 1989) from a noisy input that is relatively small (Higgs and Spain 2009). Neurons with a high gain at a specific frequency are more likely to have a phase preference at that frequency than at other frequencies.*" Note that firing "in phase" with a sinusoid for frequency-dependent gain is distinct from a spike "occurring from a sinusoidal input at a specific frequency" as mentioned above for resonance.

- A reminder of this point in the discussion as we discuss the "dynamic circuit motif" at **Lines 662-664**: "*With subthreshold resonance not observed as a general feature of L5 pyramidal cells, we sought other biophysical features that might explain why L5 cells appear to drive interlaminar theta coherence.*"

- A final note regarding the interplay between I_h, resonance, and frequency dependent gain concluding this part of the Discussion on **Lines 689-692**: "*Interpreted together, our frequency dependent gain and ZAP results suggest that I_h may not be a direct "cause" of cortical oscillations at theta (~8Hz), but rather tune RS cells to follow with great fidelity the IB output at delta (see Fig. S10 for this dynamic circuit motif).*"

It is surprising in this regard that, despite such large variances in data, that the authors felt showing n = 3 cells without any statistical analysis was appropriate. I don't even see any error bars. I do not find this experiment convincing, particularly because it's not clear how these few cells were chosen from the heterogenous population shown in Fig 4.

In our initial revisions, we were specifically asked by both Reviewers 2 and 3 to use ZD to test whether peaks in frequency dependent gain were indeed driven by I_h via specific blockade. While of course we would like to increase our "n", doing so is essentially impossible given current COVID-19 restrictions. In addition, applying ZD is a time consuming process that eliminates our ability to perform any additional experiments on a given slice, which given the limited access to human tissue is a consideration that must be taken into account when designing our experiments. Given these challenges, we feel that n=3 cells is acceptable.

In our revised manuscript, we have addressed the reviewer's suggestion to add error bars to this analysis (see **Figure 5e-f**, where the shaded area represents mean +/- one standard deviation). We have also indicated the resting membrane potential and input resistances of these three cells in the figure legend, to provide further context for how these cells fit into the broader landscape of cells that we have reported here.

2. It is remarkable that such cells as cell f or cell g shown in Fig. 1, which only reach up to layer 3 or even the border between layer 3 and 4, are presented as representative examples of layer 5 pyramidal cells that are presumed to be intact along the longitudinal axis. Cell g is further proposed to be a thick tufted layer 5 pyramidal cell (line 539), even though its morphological reconstruction simply lacks a tuft. Such features are completely unencountered in rodents and, to our knowledge, in humans as well. Note that the spiny neurons that are labeled as intact in the Allen Institute database whose electrophysiology and morphology are available (of which there are only two cells) also have visible apical dendrites reaching all the way up to layer 1, while at the same time displaying classic morphological features of neocortical layer 5 pyramidal cells.

We thank the reviewer for their careful review of the morphologies shown in Fig 1, and specifically for the comment that as cell g has no visible tuft, it should not be referred to as a putative thick-tufted cell - we have corrected this in the text on **Line 310-327** in our revised manuscript.

Below we have summarized previous reports of morphologies of human L5 pyramidal cells. In addition to the reconstructions reported in our study, there are 2 major published reports of human L5 pyramidal cell morphologies:

- Mohan et al., 2015, *Cerebral Cortex*: In this report, very few L5 pyramidal cells had dendrites reaching past L3, consistent with our study. The portion of their Figure 2 showing these L5 cells is included below for reference. As reported in this work: "To include a neuron for reconstruction, first the biocytin signal had to be dense and uniform throughout distal dendrites. Second, dendritic structures had to show minor cutting artifacts by the slicing procedure and had to be retained in the slice as much as can be expected realistically. These 2 criteria rejected about 80% of recovered neurons from reconstruction, mainly because of obvious truncation of the apical dendrite (Mohan, Verhoog et al. 2015)."
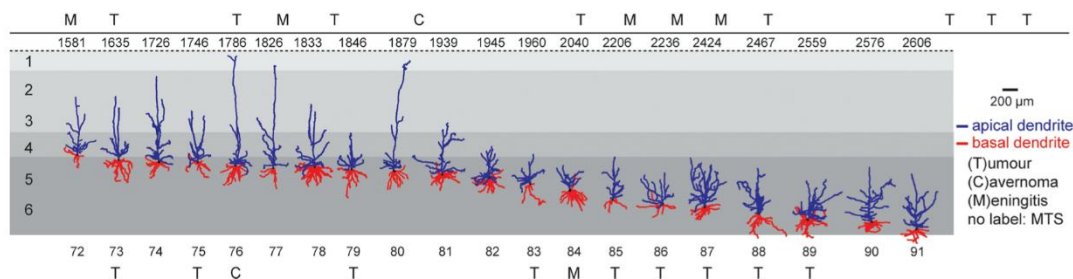

Figure 2. Dendrite gallery of human temporal cortex neurons. Representation of 91 3D reconstructed apical and basal dendrites of human temporal cortex (Brodmann area 21) arranged along somatic depth with respect to pial surface. Capitals (T, C, M) indicate that tissue was obtained from patients with subcortical tumor, cavarnoma, or meningitis. First row indicates depth in μm. Apical dendrite in blue, basal dendrite in red. Neurons not labeled originate from patients with mesiotemporal sclerosis (MTS).

- Beaulieu-Laroche et al., 2018, Cell: According to the authors of this work, each of the pyramidal cells recorded had "thick apical dendrites reaching L1". In addition, they "targeted the thickest dendrites and biggest L5 somas to isolate putative L5B neurons

(Hattox and Nelson 2007, Hay, Hill et al. 2011, Harnett, Xu et al. 2013, Harnett, Magee et al. 2015, Beaulieu-Laroche, Toloza et al. 2018)." Thus, we reason that these cells are likely to be extra-telencephalic and might not be immediately consistent with ours or those of other reports which did not specifically target recordings to the largest cells in L5. We have emphasized the distinction between ET and IT pyramidal cells in a multitude of locations in the revised manuscript, including in the Discussion **(Lines 649-652, 671-673, 630-639**) and various parts of the Results **(for example, Lines 536-543, 563-567).**

- Unpublished data of 2 L5 cells from Allen Institute: We agree with the reviewer that their 2 spiny L5 cells labeled with intact dendrites do indeed reach L1.

From these reports, and in particular the study of Mohan et al., we feel that the evidence is mixed whether all human L5 pyramidal cells have apical dendrites that reach L1. However, given the challenge of potential dendrite truncation when preparing slices containing such large cells, we cannot rule out that our representative L5 morphologies in Fig 1 have not been inadvertently truncated.

Lastly, we feel that the evidence is further mixed whether all mouse L5 pyramidal cells also have apical dendrites that reach L1. As one example, as reported recently in Adkins et al., (Adkins, Aldridge et al. 2020) and further illustrated in Scala et al., (Scala, Kobak et al. 2020)using the Patch-seq method which enables transcriptomically identifying IT-projecting L5 pyramidal cells and further reconstructing cell morphologies, in this study they report a number of IT-defined L5 pyramidal cells with apical dendrites that do not terminate in L1. See Figure 4 c, g reproduced below from this work illustrating these findings.

In the revised manuscript, we have summarized our findings and those of others in **Lines 321-327**.*"While we note that our human L5 morphologies are different from those reported by Beaulieu-Laroche et al. (Beaulieu-Laroche, Toloza et al. 2018) that targeted rare thick-tufted L5 pyramidal cells (Hodge, Bakken et al. 2019) with tufts reaching into L1 (Ramaswamy and Markram 2015), our cell morphologies are consistent with other previous reports that relatively few L5 neurons have dendrites extending past L3 (Mohan, Verhoog et al. 2015). Additionally, given the challenge of potential dendrite truncation when preparing slices containing such large cells (Mohan, Verhoog et al. 2015), it is possible that our representative L5 morphologies in Figure 1 have been inadvertently truncated (Mohan, Verhoog et al. 2015). However, we only observed visible truncation in one branch of one cell (the largest cell, cell g) and no obvious truncation in the other cells shown in Figure 1"*.

In addition, it is worthwhile to note here that the presence of spines alone is not a guarantee for a cell to be pyramidal or even excitatory, and interneurons including the sparsely spiny interneurons are also found more frequently in primates than in rodents (Kawaguchi & Kubota, 1993; DeFelipe, 2011). Whether the apical dendrites of cells recorded are intact is of much importance for the current study, not only as it will affect passive membrane properties but more importantly because ion channels including HCN channels are known to be expressed in steep gradients along the somatodendritic axis (Lorincz et al., 2002).

We agree that the presence or absence of apical dendrites is important for our study, especially as it relates to the somatodendritic distribution of HCN channels. In our revised manuscript, we now discuss at **Lines 706-712** how inadvertent dendrite truncation might impact properties that are thought to arise from I_h.

It is also unclear from the manuscript how the authors excluded the non-fast-spiking interneurons (which coincidentally have high input resistance) from analysis, which are much less obviously distinguishable from pyramidal cells by electrophysiology alone to the untrained eye. The authors describe that putative interneurons were identified by spike properties such as action potential width, maximum firing rate, and strong afterhyperpolarization, all of which are well-suited criteria for fast-spiking interneurons such as those shown as representative examples, but much less effective for identifying non-fast-spiking interneurons (a population which also overlaps with the aforementioned sparsely spiny interneurons such as calbindin-expressing interneurons in the deeper layers of the neocortex).

Following the comments of this reviewer and others, in our prior revision we undertook a systematic effort to carefully review electrophysiological data from each of the recorded neurons in our prior analysis. In particular, in this re-analysis we were able to identify and remove from our primary analysis a number of putative interneurons, i.e. those with characteristics reminiscent of more narrow-spiking interneurons. Using similar approaches, Torres-Gomez et al. showed that intracellular spike waveform features can sufficiently distinguish PV and SST cells from pyramidal cells (see Fig 5 in Torres-Gomez et al.). (Torres-Gomez, Blonde et al. 2020)

However, as the reviewer states (and further supported by the work in Torres-Gomez et al.), it is not possible to use intracellular electrophysiological features alone to further separate interneurons with more broad-spiking spiking features, such as VIP interneurons, from pyramidal cells. We acknowledge this as a limitation of our analysis and note this would be a limitation in any analysis where pyramidal cell identities were not confirmed post-hoc using morphological reconstructions or another methodology. However, VIP (and other CGE-derived) interneurons are relatively infrequent, making up approximately 5% of all neurons in L5 of the human neocortex. In the **new Fig S9** we have replotted cell type proportions based on transcriptomics data from MTG from Hodge et al. (Hodge, Bakken et al. 2019). Therefore, if VIP and other CGE-derived cells are present in our dataset, it is reasonable to expect that their prevalence would be somewhat rare. In our revised manuscript, we have explicitly addressed these issues in the Methods at **Lines 166-172**.

3. The authors continue to place irrelevant and inappropriate references in attempts to support their otherwise unfounded claims, even after having been corrected for numerous such instances from their previous manuscript. For example, they state that "human neocortical circuits demonstrate […] different short-term plasticity rules, compared to neocortical circuits in rodents"; setting aside the ambiguity on exactly what type of synapses the authors are referring to here, one of the two cited papers is entirely on rhythmic firing in rat layer 5 pyramidal cells, while the other is about timing rules for spike timing dependent plasticity in human hippocampal (not neocortical) neurons. Neither study addresses or even mentions short-term plasticity. Another example is their statement that "Ih contribute[s] to low pass filtering properties of pyramidal cells", which first of all may not be technically correct under normal conditions; second, seems to contradict the authors' own data (Fig. 7F; S2A); and third, inappropriately references a study that certainly does not make any such claim.

While we took care to address this issue previously (and thank the reviewer for pointing out specific instances in their prior review), we apologize that inappropriate citations persisted after our initial revisions. We thank the reviewer for pointing out these 3 potentially erroneous citations. **We have corrected these specific citations and the corresponding text**, and also have rigorously gone through the remaining citations in an effort to ensure that no additional errors of this kind persist in our revised manuscript.

4. It is disturbing that the authors were able to find and remove significant parts of their original data that were supposedly included in error but went unnoticed, sometimes resulting in revoking their original conclusions (i.e. strong correlation of Ih and patient age). Taking this into account with the other inconsistencies and methodological concerns that are still present, it is challenging to come away from this paper with any strong insight about human neurons and how their intrinsic properties may contribute to brain level oscillations.

We again thank the reviewer for their comments in our first round of review, alerting us to potential data curation issues in our initial dataset. Upon receiving these comments from this and the other reviewers, we made our best attempts to systematically review our data and reconcile our findings with the past literature.

While we understand and appreciate the reviewer's concerns regarding this reanalysis, we maintain that this serves to strengthen the quality of our dataset and the resulting conclusions. We note that this opinion appears to be shared by Reviewer #1, as quoted in the most recent round of review: "The authors have done a nice job of being responsive and open to the reviewer concerns and addressing the major issues raised. *I appreciate the willingness of the authors to acknowledge various flaws that were either directly called out or discovered on their own in the course of revision. The corrections and new experimental data collected were quite important and no doubt have strengthened the manuscript over the original submission. I acknowledge that quite a lot of new experimental and analysis work was completed for this revision*" (emphasis added).

We emphasize that our initial argument regarding patient age was a relatively minor part of our initial submission. As such, we have elected to remove this analysis entirely in the revised manuscript. We do not feel that this considerably affects the quality or novelty of our manuscript.

We feel that the "other inconsistencies and methodological concerns" have been addressed (see our response to Main Points 2 and 5), which should mitigate any concerns about the quality of our experimental data.

5. The unexplained variance in basic physiological properties continues to be a subject of concern. The RMP for L2/3 neurons spans from -60 to -80 mV; for L5 it's -80 to -55 mV. The authors acknowledge this may be either biological variation or an experimental confound but do nothing further to try to resolve this. If the authors performed a set of similar experiments on mouse or rat cortical neurons in L2/3 or L5 under the conventional, highly stereotyped conditions of brain slice preparation, would they still see this level of variance? How much of the variance in this manuscript is experimenter-driven quality control in terms of slice preparation and patch-clamp prowess and how much is a result of other forces, like real biological variability and/or human brain tissue condition?

We do not agree with the reviewer that the ranges of RMP are inconsistent with what one would expect. In fact, there are numerous examples in the human literature that correspond with the level of variability seen in our study, which we highlight below:

- For comparison: in Ting et al. 2018, *Scientific Reports*, an RMP range between approximately -60 and -80 mV is reported in L2 and L3 human pyramidal cells (see figure below, the relevant portion of Figure 3 from that work) (Ting, Kalmbach et al. 2018).
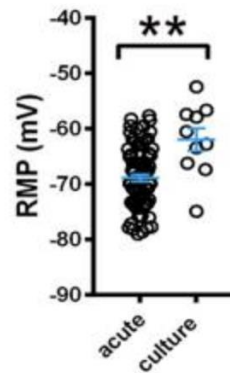

- In the work of Kalmbach et al., 2018, *Neuron* (see relevant portion of their Figure 2 below), similar variability is seen in the RMP amongst the human L2 and L3 neurons.(Kalmbach, Buchin et al. 2018).
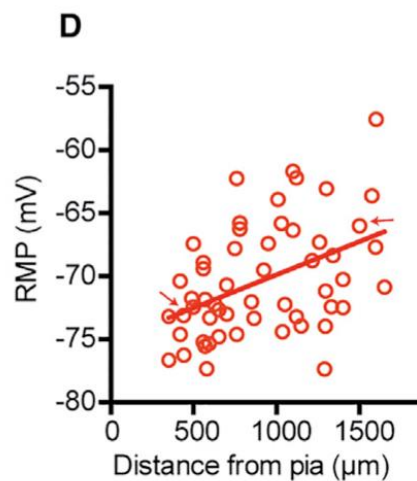

- Similar variability in the RMP is seen amongst the human L2 and L3 neurons characterized by (Berg, Sorensen et al. 2020).(see figure below, the relevant portion of extend data Figure 5 from that work).
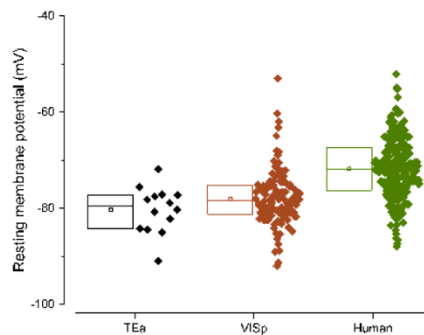
- While the work of (Beaulieu-Laroche, Toloza et al. 2018) shows less variability in RMP (approximately -70 to -55 mV, see relevant portion of Figure S1 taken from that work below), one possible explanation is that as they targeted the largest L5 pyramidal cells, these cells might reflect a more homogeneous population than the set of L5 cells targeted here **(a point that, as noted above, we have emphasized in our revised manuscript)**.
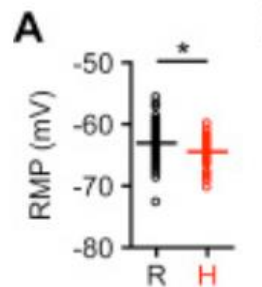
On this point, it is worth noting that recordings from the same work in L2&3 (included as Supplemental Figure 2, the relevant portion of which is included below) shows a slightly increased range of RMPs, which is similar to the variability reported in our work.
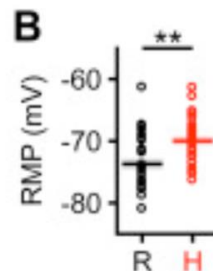
Speaking of which, are there inclusion criteria for human brain samples? One can imagine that depending on specifics of the surgery and the patient, the tissue could be in very different states, potentially contributing to the health on individual neurons and their resting membrane potential. How do the authors deal with this?

Our inclusion criteria for human brain samples is provided in the Methods section on **Lines 94-96** in the revised manuscript. On general issues related to the quality of human surgical tissue, we point the reviewer to **Lines 713-724** of the revised manuscript. In short, we have taken care to acknowledge the challenges and caveats of making recordings from human surgical tissue, where tissue quality cannot be as precisely controlled as with rodent recordings.

Additionally we note that the existing human work by (Mohan, Verhoog et al. 2015, Kalmbach, Buchin et al. 2018) both delve into this topic in great detail, noting the necessary conventions used and accommodations afforded in the study of human neurons, given our limited access to human tissue and the resulting inability to "control" for as many factors as can be done in analogous rodent studies (see specifically the section entitled "Caveats of Human Tissue" in the Discussion of the Mohan et al. work). We explicitly cite these works to make this point on **Line 718** of the revised manuscript.

In general, as illustrated above for RMP values and in our prior revision for input resistance values (see Fig S1), we feel that our data are largely consistent with those reported previously. We thank this reviewer for encouraging us to perform this comparison as systematically and rigorously as possible. Following these comparisons, we now feel confident that unavoidable issues, such as surgical tissue quality, do not significantly compromise the results reported here.

In summary, is it not clear what the authors want the reader to conclude at the end of this manuscript. The experiments and analysis have all largely already been performed in human neurons, and the attempt to connect these cellular properties to oscillatory dynamics, as has already been done in rodents, is weak. It is thus hard to understand how the current work will have a substantial impact in the field.

Premised on the hypothesized functional differences in superficial and deep layer neurons as evidenced by collective neuronal dynamics, this work clearly addresses what we set out in our introduction: to explore the differences between human deep and superficial layer neurons in pursuit of a well-supported hypothesis explaining their functional differences. Through our explorations we show a large number of unique findings, and utilize complementary analysis to disentangle the functional implications of the differences between superficial and deep layer human neurons not previously done in human or rodent work.

We thus believe our work is impactful, a perspective we note is shared by Reviewer 1 and the original Reviewer 3, especially considering the posited relevance of using human inspired data to build human inspired models of the brain. We further note that, outside the subjective arguments of "impact", *we can objectively state that our manuscript adds a valuable missing perspective on human L5 presumed-IT cells*. According to recent transcriptomic evidence (Hodge, Bakken et al. 2019), IT cells are expected to be far more prevalent in human L5 than the ET/ thick-tufted cells that have been recently studied in (Beaulieu-Laroche, Toloza et al. 2018). This is included in our manuscript as the new **Figure S9.** We also feel that, given the rare opportunity to perform such experiments in live human tissue, it is extremely valuable to compare and contrast

our findings with those reported by other groups— *this is a strength of our manuscript, not a weakness.*

In conclusion, *we do believe our work will have substantial impact in the field*, not only by improving our understanding of the intrinsic properties of human neurons, but also in providing one of the first explorations of the impact these properties may have on the oscillatory properties of the human neocortex.

Original Reviewer #3 (and responses from the initial round of revisions):

This is a thorough, careful and thoughtful MS quantifying the distribution of Ih in human – mainly anterior temporal – neocortex by layer. I completely concur with the ethos behind the study – that modelling the human brain demands constraint by human data and, for this reason alone, **the paper constitutes a valuable reference work and so to me, deserves publication**.
I do have a couple of issues with the MS as it stands though. **The comments below are, hopefully, easily dealt with and are intended merely as suggestions to improve the MS**.

We have added emphasis to two key points from the original Reviewer #3's comments, which we feel speak to value of our manuscript (and perhaps might help address some of these concerns raised by Reviewer #2).

1) I see why the authors 'hang' their findings on the human theta rhythm. But I don't think its valid given precedents in rodent literature. As they argue in the discussion, there are multiple mechanisms that can underlie neuronal rhythmicity at theta frequency. Ih is almost certainly not one of them in neocortex. Hippocampal theta cannot reliably be considered the same phenomenon, the frequencies are different for a start. Here there is a dependence on Ih but it lies principally in the behaviour of a subset of interneurons and the neocortical equivalents were not examined in the present MS. In addition, Ih is exquisitely dependent on neuromodulatory state: Theta is mainly seen in non-invasive human recordings in the wake state but, in this condition, multiple wake-associated neuromodulators all act to reduce Ih considerably (e.g. Ach, Orexin/hypocretin etc.). What Ih IS critically involved in is rebound following synaptic inhibition. Thus it plays a crucial role in delayed responses to sensory input (the 'off' response) and 'anodal break' spiking seen in mismatch responses.

We thank the reviewer for this thorough and positive comment. We indeed agree with the reviewer's analysis that theta rhythmicity is a complex dynamic, both at the cellular and network level, that is driven by a wide variety of factors beyond I_h, and that there are important differences between human and rodent theta rhythms. We have thus endeavored to downplay the emphasis on theta resonance. Our ZD-7288 (I_h blocker) experiments firmly establish that the low frequency peaks in G(f) particularly in the delta frequency are dependent on I_h. In light of these new results, we interpret our previous findings of interlaminar coherence at theta frequency (4-8Hz), as arising from the dynamic interaction between IB cells (that burst at delta), and RS cells (which are the predominant cell-type we likely recorded from) that receive delta frequency input from IB cells to which they are particularly tuned to (peak in delay in G(f)), which then discharge at theta frequency

(and possibly in part the theta frequency peak in G(f)). Please see response to last comment for a continuation of this discussion (**Figure S10 in revised manuscript**).

2) The data is presented with commendable clarity, but consequently suggests multimodal distributions in a number of the intrinsic cell properties measured. This is discussed briefly (L2 vs L3 for example) but the main subdivision of cell type I know of that manifests in part as sag amplitude differences is between L5a and L5b. The authors have gathered an impressive set of data so I wonder if it is possible to stratify L2/3 and L5 cell types further on the basis of other intrinsic properties (slow AHP, burst generation, after depolarisation strength etc.) This may help to 'clean up' the often very broad distributions in some of the metrics.

We thank the reviewer for this excellent comment. We point the reviewer to our analysis in what is now Fig 6, which attempts to address some of the large degree in heterogeneity within cells from the same cortical layer.

We also note that we address this topic in our responses to similar comments from the other reviewers: specifically, we have addressed some of this increased variability by identifying interneurons and distinct experimental protocols that were mistakenly included in our data set, while noting that some level of increased heterogeneity in these cells (relative to the rodent setting) is to be expected based on the emerging human cortical cell typing literature.

2 minor points:
3) Lack of observed resonance on somatic recordings with patch electrodes is not surprising. Patch solutions dialyse cytosol hugely and thus interfere with many intrinsic conductances. In addition, the distribution of Ih in neurons is not uniform and resonance can be seen in dendritic recordings in a given cell type when it appears completely absent in somatic recording.

We thank the reviewer for noting these potentials confounds in our subthreshold resonance data. We have now added a paragraph to our discussion within our limitations section to contextualize how experimental confounds, such as dendrite cutting or cytosol dialysis, might influence some of our ability to observe subthreshold resonance in these data.

4) The MS data does agree with the Carracedo data in terms of layers showing most theta (discussion). In that paper the field theta was mainly manifest in superficial layers but the origin of the synaptic activity underlying this was exclusively intrinsic theta activity in a subpopulation of L5 cells. See point 1 above though, this theta was not Ih-dependent.

We thank the reviewer for this excellent comment and insights. We fully agree that I_h is likely not directly responsible for the theta frequency activity in local circuitry. We do however argue in line with the (Carracedo, Kjeldsen et al. 2013) work, that it is indirectly complicit in theta generation given our new experiments added to this MS, that show that the delta peak (~2Hz) in G(f), and less so the theta peak (~7Hz) are dependent on I_h (see Figure 5e-f). From the (Carracedo, Kjeldsen et al. 2013) paper it was the RS cells that generated theta, likely the cell-type that primarily contributed to G(f) (Figure 5b). We speculate that they did not observe theta in the deep

layers, since human circuits appear to amplify local activity relative to rodent cortex (Molnár, Oláh et al. 2008) which might make theta more prominent in human L5. Conversely, we did not observe delta activity in our previous in-vitro recordings, possibly due to the paucity of IB cells in human middle temporal gyrus cortex (Hodge, Bakken et al. 2019). Our data as well provides a putative cellular mechanism (peaks in G(f)) underlying these population activities. We have schematized these ideas below and include it as a supplementary **Figure (S10)** and clarified this in the discussion.

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Reviewer #1 (Remarks to the Author):

I am satisfied with the revisions by the authors. I recommend that the manuscript should be accepted in present form for publication in Nature Communications. The revised Figure material that was more appropriate for the supplement has been moved to the supplement, as requested, with additional changes to the corresponding text. The authors have made their best attempt to address concerns of all authors. The human slice physiology presented in this study is imperfect but is honestly represented and the authors do their due diligence in trying to reconcile their findings with other studies of similar scope. For example, the response about the wider than anticipated range of RMP values for human pyramidal neurons was clearly answered with compelling evidence that this is within the expected range for human cortical pyramidal neurons. The addition of figure S9 is really helpful to explain the sampling issue and how abundant L2/3 IT pyramidal neurons are in contrast to more rare L5 types, as well as how rare the undersampled L5 ET or thick-tufted neurons type is in human temporal cortex. This is a limitation of the approach of recording in unlabeled tissue slices and the authors have reasonably acknowledged the limitation. I also appreciated the points about intactness of L5 pyramidal neurons and absence of apical tufts. It was compelling and interesting to compare to examples from the largest study of human neuron reconstructions to date (Mohan et al) as well as to the mouse Patch-seq study from Tolias lab where even some mouse L5 IT neurons don't have clear apical tufts. It remains unresolved if L5 IT neurons in human (and mouse) cortex can exist without an apical tuft, if this feature varies by brain region or finer subtypes, and what is the functional significance of tufted vs non-tufted L5 neurons (although there are obvious implications). This will be a fascinating topic for future investigation.

I strongly believe that the novelty is an important factor, but there is ample room for incremental advances and more than just a handful of publications on physiology and morphology of human cortical pyramidal neurons. We need more work like this published, not less. Each study is informative to the field about how to make advances and increase the rigor of the work for future investigations in spite of the major challenges to doing human brain slice recordings.

Jonathan T. Ting